

The quiet side of technology

How many people understand what the state does on our behalf in prisons, in conflicts such as that in Northern Ireland, or in the murky world of demonstrations, pickets and strikes? And how well aware are scientists and technologists of the uses to which the armed forces, the police and the prison service put science and technology in pursuit of domestic policies? There is general and widespread ignorance of these subjects, and so the appearance of *The Technology of Political Control*, by Carol Ackroyd, Karen Margolis, Jonathan Rosenhead and Tim Shallice (Penguin £1.25), should have been welcomed as a guide to an uncharted area. But the book, which stems from investigations organised by the British Society for Social Responsibility in Science (BSSRS), is too ill-focused, full of ideology and lacking in constructive thinking to have anything like a major impact on the scientist who knows little and wants to know more.

The authors themselves write that their approach 'may seem a curious amalgam of technological exposé and political analysis' and indeed it is. The first half of the book is devoted to a rambling analysis of the steps by which Britain could move towards being a 'Strong State', obsessed less with the threat from without than with that from within as it lays plans in anticipation of social conflict. Such a strong state would have learned a lot from the Northern Ireland experience and would practise the sort of low-intensity operations described by Frank Kitson in a book of that name. The army, more closely integrated with the police, would play a significant role in putting down insurgency.

If you believe, as do the authors, that the major function of the British legal system is to protect property, that the events of the twentieth century are increasingly posing the question of socialism or barbarism and that any new working class revolution will have to ensure that ultimate power resides with the working class, then you will probably accept their analysis. A less class-conscious view of the world would probably not reveal quite the same trends, but that is not the point. The point is, as the authors insist, that they are writing as 'concerned scientists and committed socialists'. The actual technology of political control is therefore at least as important.

The technology occupies the second half of the book and amongst other things includes phone-tapping, bugging, computers, sensing equipment, riot control techniques, method of psychological torture and misuse of psychiatry. It is described as a 'vast and grim catalogue available to

Western governments'. But the detail contains few surprises. Indeed, it is one of the disappointments of the book that it draws so widely on newspaper cuttings and so little on detailed research by the authors. Given the grass roots contacts BSSRS has been developing in the past few years, such research surely ought not to have been impossible.

Take the example of the computer. The police and the army already use computers as data banks, of course, and these uses are described. It takes little imagination to spot that computers may have a broader role to play in stitching together low grade information: as one Pentagon enthusiast put it some years ago, given an adequate computer network in Northern Ireland, one could put all sorts of information together, such as that Mrs O'Reilly hasn't gone out for her daily walk to the baker's, and come up with suggestive patterns of use to the security forces. But if this is true and it has been done, it poses all sorts of questions about the privacy of the individual. The authors only very tangentially pick up this issue, either technically or in its implications, and give little sign of persistent enquiry. So it seems with many other technical issues.

To the authors of this book, modern technology in the service of the state's law enforcement agencies is clearly an affront because it is or will be used to keep down working-class-generated insurgency and revolution. But what do they say scientists should do? It is here that the book is at its weakest and briefest. The answer seems to be that they should work politically for the abolition of the capitalist state and the establishment of a workers' militia in place of the capitalist army.

Those who take a less extreme view will be more likely to say that many of the problems of technology and political control are not amenable to such neat solutions. In Northern Ireland there is now plenty of technology on both sides, none of it particularly palatable, and talk of working-class control is meaningless. In other less strife-ridden parts of the country the unspectacular recipe is eternal vigilance. Technology does indeed have its role to play in law enforcement, and law enforcement agencies would be remiss if they did not use technology to the full. But the question is at what place use becomes abuse, reasonable enquiry becomes harassment, monitoring becomes intrusion. This can only be solved on a case-by-case basis, with at least some sort of trust in political institutions. The real need is for scientists who are involved in military and police research to be able to speak up when they see the balance shifting in an unsavoury direction. □



Directing Cuba's science

Mahindra Naraine, recently in the Caribbean, assesses the achievements of Cuba's science policy

IN FEBRUARY of last year, Cuba proclaimed a new constitution and established a new governmental system which transformed its existing central administration: ten months later, in December, the Council of Ministers—effectively the new Cuban government—was appointed by the National Assembly. The country was no longer ruled by decree, and elections had been held. The changes, marking the fourth phase of Cuba's modern history (see box), are now having their impact on the country's science and technology policy.

The institutional and infrastructural framework that the new order supercedes had a chequered record. It was characterised by the conscious promotion of science and technology—training personnel, creating research institutions, establishing means of international collaboration and starting research projects. Thus, in 1962 a new Academy of Sciences was created. In conjunction with the Soviet Academy of Sciences it assessed the country's natural resources, and, had as its first priority the creation of a soils map and national atlas. Other institutes were also established: the Institute for Sugar Cane Research, the Meteorological Institute, the Institute for Scientific and Technical Documentation and the Institute of Nuclear Research. In addition, the Ministry of Industry decided to set up special institutes to seek immediate or long term policies for the use of natural resources; these included institutions for the study of sugar cane by-products, mineral research, chemical research, meteorological research, standards and metrology.

In 1965 the National Centre for Scientific Research (CNIC) was created to carry out research in several fields, especially in the biological sciences. Most of its work is basic research, and it enjoys close associations with the various institutions and with the University of Havana. But although a few coordinating bodies were established—Councils of Agricultural Research, Sugar Research, Geology and Geophysics Research and so on—there was still no central directing body for science and technology. By 1974 the Cubans felt that they had a 'critical mass' of personnel and resources oriented towards research and development and that their socio-

economic development was solid enough as a base for further work.

There was good reason for this. The investment in the universities in the 1960s began to bear fruit in the 1970s with a growing volume of research and increasing manpower. There are now more than one hundred basic research centres of varying size including research institutes, experimental stations and specialised laboratories, in which there are over 21,000 technical workers (about 20% of them university graduates); expenditure in 1975 on the basic research centres was \$75 million. In 1957 there were an estimated 4,000 engineers and scientists and 5,000 middle-level technicians; in 1975 there were some 30,000 university graduates, about half of them scientists and technologists. University enrolment has grown from 15,000 (1959) to 30,000 (1970) to 83,000 (1975); the plan envisages 140,000 in higher education by 1980. In 1974 the breakdown by disciplines was:

Science and technology	52.8%
Agriculture	16.3%
Medicine	18.3%
Education	1.8%
Economics	7.0%
Social sciences	3.8%

In 1958 only 11% of the students were enrolled in science and technology. According to Cuban figures, there are 20 graduates and technicians per thousand of population. This means there are some 170,000 technicians in the country.

Highest priority

The Cubans have given education the highest priority, spending \$874.6 million in 1975. The 1976–80 plan provides for 1.7 million in primary school in 1980, and 1 million in secondary school. There are four universities, and in the different provinces there are also 'university colleges' which are attached to one of the universities. 'University units' are centres of production, services and research where workers can carry out productive activities and take classes. University research is to a great extent carried out in teaching departments as an integral part of the students' science and technology training and the education of the staff. Some faculties have developed centres around specific topics, where teachers are assisted by researchers working in nearby schools and where post-graduates are trained. Some centres,

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for example the Centre for Digital Research and the Institute of Animal Science, do only research and post-graduate training; this type of centre is not directly dependent on the faculty or schools, and aims to train quality personnel and develop research.

With such advances, it was by 1974 time for more changes. In June of that year the government decided to establish a National Council of Science and Technology (CNCT) to manage the country's science and technology policy. Central management was deemed necessary to avoid publication, reduce cost, manage international collaboration and to coordinate the work of the various institutes with socio-economic development. Indeed, in establishing CNCT it was explicitly stated that a national science policy was a political not a technical decision.

CNCT was set up as an organ of the Council of Ministers with its President being the Vice-Premier of Education, Science and Culture. One of its vice-presidents was the President of the Academy of Sciences. The immediate task of CNCT was to draw up the national science and technology policy; once this was approved by the Council of Ministers, it was expected to control the execution of the plan and to propose practical application of the results obtained. The CNCT itself, which did not administer any research and development centre, had three main parts:

1. Presidential Council (President, Vice-Presidents, Secretary General and Directors.)

2. Advisory Committee (Members of the presidential council; well known scientists from different organisations and institutions; technical ministers and or vice-ministers concerned with research sectors and sub-sectors of research departments in universities; directors of the major research institutions.)

3. Scientific councils (Set up according to the nature and needs of the managing bodies, they are intended to bring together the most famous specialists in the relevant discipline independently of the organisations where they usually work.)

Close collaboration with the Central Planning Authority (JUCE-PLAN) and with other bodies was necessary to achieve proper integration of science and technological plans with the socio-economic and cultural development of the country and to ensure that the science and technology plan would be one of the categories of the national economic plan. The 1976-80 economic plan was approved at the first Congress of the Cuban Communist Party in December 1975, and has three aims: to increase and diversify agricultural production to make the country self sufficient in food; to increase industrialisation; and to increase the production of sugar and nickel to pay for imports.

In agriculture the main areas of expansion will be rice, vegetables, citrus, poultry, fisheries and livestock. Industrial production will be increased in all areas, particularly fertilisers, steel, cement, paper and agricultural machinery. It is planned to produce about 600 cane harvesters a year from a Cuban design; this will reduce the workforce in the sector from about 370,000 to 50,000 by 1980. A nuclear reactor with capacity 880 MW will be built with Soviet assistance; in 1975 Cuban electrical generating capacity was 6.5×10^{12} W (about 700 kWh per inhabitant) but this is expected to increase by 35% over the next five years.

In 1959 sugar constituted 80% of Cuban exports and it held a similar position in 1970. In the future it is expected that the extensive Cuban nickel deposits will take over the role of main exchange earner, hence the 300% proposed expansion in nickel production.

CNCT plan

The CNCT drew up a plan for 1976-80 to link up with the national economic plan, slowly integrating indigenous research with technology transfer. Technology transfer is considered by Cubans to be indispensable to rapid economic development, but for it to be effective it must be adapted to local conditions and that means a large research and development base is essential. The CNCT plan focuses on the exploitation of Cuba's extensive laterite deposits; the application of nuclear technology and solar energy; the development of the electronics industry (to produce 100,000 television sets and 300,000 radios per year) and the development of computing and information systems (Cuban designed mini-computers are now being manufactured with Japanese components—under a Soviet-Cuban agreement, Cuba will provide the Comecon countries with certain components); the use of local products; and agriculture,

Cuban chronicle

Over the past hundred years Cuba has undergone three major transformations, and the development of its science and technology policy has tended to reflect this historical development.

The second half of the nineteenth century, ending with the first US intervention from 1898-1902, coincided with the rise of Cuban national consciousness and the struggle against Spanish colonialism. The first Cuban Academy of Sciences was founded in 1860, immediately prior to the Ten Years War, by about thirty European-trained academics, many of whom made contributions to world science. Attempts to introduce scientific and technical methods to Cuban agriculture, then in a primitive state, were unsuccessful; colonial conditions meant the Academy was 'peripheralised', and new development stemmed from technology transfers rather than indigenous sciences. By 1895 there were 1,186 students in institutions of secondary education—0.7 per thousand of population; the subjects studied were mainly Greek, Latin and rhetoric.

Cubans view the period beginning in 1902 and ending in 1958 as "the period of the neo-colonial republic marked by the presence of American imperialism." Local scientific activity was minimal and there were few research centres. The Academy of Sciences suffered a slow decline: by 1959 its yearly state budget was \$600 and it did not possess a librarian. It was attached to the Ministry of Justice, while the Geographical Society of Cuba fell under the Ministry of State and the National Observatory was part of the Ministry of Sea and War. By 1958 15,000 students were enrolled in higher education, with 11% studying science and technology. About 60,000 students were in secondary education, representing about 10 per thousand of population. American missions in 1937 and 1950 commented on the paucity of technicians, the scientific and technological backwardness of the country and especially in the sugar industry, and the lack of facilities for research at the University of Havana.

The period was one of great political instability and culminated in the Batista dictatorship. The plantation economy, dependent on sugar for more than 80% of exports, was heavily reliant upon the US economy both in terms of trade and

direct investment. The urban/rural dualism intensified, and by 1958 Cuba had half a million unemployed and two million illiterates and semi-literates in a population of 6.5 million. The period is seen as one of increasing cultural domination with continual attempts to belittle Cuban achievements. The example is cited of the recognition given to the American Dr Walter Reed as the discoverer of the carrier of yellow fever; in Cuba the Cuban Academician Dr Carlos Finlay is considered to have deciphered the problem.

The successful revolution of Fidel Castro and his followers in 1959 marked the start of Cuba's third period. Influenced by Soviet industrialisation, Castro and particularly Ché Guevara felt that a similar programme was necessary for the Cuban revolution to succeed. Science and technology were therefore recognised as critical factors in social and economic development, and in spite of a critical resource shortage, human and material resources were allocated for the creation of research centres to meet the requirements of the development plan.

The development plan which emerged for 1961-63 followed eighteen months characterised no less by chaos and government measures to gain control over all aspects of Cuban society, than by a programme of agrarian reform and a campaign to eradicate illiteracy. It was drawn up by Guevara and was intended to transform the Cuban economy from an agricultural to an industrial structure. It was expected to accelerate development on many fronts by means of import substitutions with emphasis on heavy industry. The programme exceeded Cuba's technical, administrative and resource capabilities; it ended as a failure.

The second growth strategy from 1964-75 reoriented previous plans, with their emphasis on agriculture, to centre on sugar and exports. Industrialisation remained the long term objective, but it was to be a gradual process with immediate expansion on a few fronts, such as cement manufacture, fertilisers and steel. Consequently, the science and technology infrastructure continued to expand, particularly in the training of personnel and in the establishment of research centres (see main story).

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livestock and fisheries. Over 100 problem areas have been identified for research and development activity.

Under the most recent changes, however, the National Scientific and Technological Council (CNCT) has disappeared. Its duties have been taken over by the State Committee for Science and Technology (CECT), whose head is a member of the Council of Ministers. This committee is the agency in charge of directing, coordinating and supervising the implementation of state and government policy in science and technology. It has to advise the government, prepare science policy, supervise research, establish research centres, and manage the science and technology information system.

The Academy of Sciences is now classed as an institute that is a central state agency, but its director will not be a member of the Council of Ministers. It has been charged with the drawing up, supervision and implementation of basic research and with being the link with foreign organisation. The various other technical ministries, covering, for example, the chemical industry, and mines and geology, will continue to have their own laboratories and do their own industrially oriented research. The work of all such ministries, however, will be coordinated by the CECT,

which will also link up with the Academy of Sciences, the Ministry of Higher Education and the Ministry of Economic Collaboration.

Growing role

Cuba's international role is now growing; it is unusual to find a country so little endowed with natural resources such a large source of aid. Cuba's aid essentially comes from the technical expertise that is the result of its massive investment in scientific and technological education. The success has been little short of phenomenal, and it is estimated that there are more experts in the developing countries from Cuba than from any other country. But it is well known that Cuba has not achieved this by its own means. Since 1960 it has been drawn into the Soviet orbit; in 1970 the Soviet-Cuban Commission for Economic, Scientific and Technical Cooperation was formed, and in 1972 Cuba joined Comecon. Cuban development has been underwritten by the Soviets to the estimated sum of \$5 billion since 1959. The full contribution of the Comecon countries is hard to assess, for apart from credits they provided expertise in the early years and also trained Cuban personnel. In 1969 as much as 20% of the scientific and technical staff engaged in research

and development in Cuba were foreign but this manpower assistance declined as the Cuban investments in education began to produce results around 1970.

An important instrument in Cuba's new international role is the State Committee for Economic Collaboration (formerly the National Commission for Economic, Scientific and Technical Collaboration). In coordination with the State Committee for Science and Technology, it plans and administers foreign technical aid. As the relentless industrialisation drive continues, Cuba's voice is growing more important in the 'North-South' dialogue. Along with India and Yugoslavia she has been one of the leading advocates of greater trade between the underdeveloped countries. For a country that in the next decade expects to be the most industrialised of that group, it sounds like good business. Mexico and Cuba have already designed sugar mills and equipment for sale to the developing nations.

How far Cuba will be able to continue this programme and supply these countries is uncertain. What is certain is that a massive investment in science and technology has meant great political and economic gains. Castro was indeed prophetic: "The future of this country," he said in 1960, "must be the future of men of science." □

USA

Feasible, sound and attractive

A new report on the prospects for solar energy was published last week. Colin Norman reports from Washington

GIVEN sufficient political and economic support, solar energy technologies could supply as much as 40% of the world's energy needs by the turn of the century, and a startling 75% by the year 2025, according to a report published last week by the Worldwatch Institute, a small think-tank concerned with global problems. Such a major shift from fossil fuels to renewable energy resources "would require an unprecedented worldwide commitment of resources and talent", but the social and economic benefits "would far outweigh the costs and difficulties", claims Denis Hayes, who wrote the report*.

Though a multitude of enthusiastic articles and studies have been written

in the past few years to extol the virtues of solar power, the worldwatch report is already attracting considerable attention. For one thing, it was published while the Carter Administration was in the midst of putting together a long-awaited energy policy for the United States, a policy which is expected to place increased emphasis on renewable energy resources. It also breaks new ground in analysing the worldwide potential for solar power, while most other studies have concentrated on solar prospects in a single country. And its upbeat conclusions are also tempered by some realism about the political obstacles along the road to a solar powered world.

Hayes begins by noting that a transition from oil and gas to alternative power sources is inevitable, given the fact that production of oil and gas is expected to peak in the 1980s and that the price of those fuels is already beginning to increase by leaps and bounds. He argues that a switch to coal to provide energy needs over the next 50 years would result in "an absolutely

intractable problem" of massive increases in atmospheric carbon dioxide, that a switch to nuclear power would require building so many nuclear reactors and recycling so much plutonium that "such a prospect cannot sanely be greeted with equanimity", and that fusion power is a long distance, unproven technology. That leaves the solar options, which Hayes defines as wind, falling water, biomass and direct sunlight.

Though Hayes is quick to acknowledge that most solar technologies are hard put to compete economically with conventional fuels at current prices, he argues that many alternative power sources have environmental costs and hidden subsidies which are not usually included in economic comparisons. As those costs are not internalised and as oil and gas prices spiral upwards, solar energy will become increasingly attractive, Hayes suggests.

Straightforward comparisons between solar power and conventional fuels also neglect an important factor: solar energy is not just more of the same. "Most energy decisions are based on the naive assumption that competing sources are neutral and interchangeable", Hayes notes, but solar technol-

**Energy: The Solar Prospect* (Worldwatch Institute, 1776 Mass Avenue NW, Washington DC 20036, \$2).

ogies offer a highly decentralised and flexible power source which, if widely used, would result in a very different world from one powered chiefly by large central generating stations running on coal or nuclear fission. Solar technologies, Hayes argues, "are more compatible than centralised technologies with social equity, freedom and cultural pluralism".

Hayes's basic message, therefore, is that a transition to a solar era "would be technically feasible, economically sound, and environmentally attractive". Furthermore, "the most intriguing aspect of a solar transition might lie in its social and political ramifications". A constant refrain running through Hayes's study is that many relatively unsophisticated solar technologies are already sufficiently developed to be put to use, either alone or in tandem.

On solar heating and cooling, for example, he argues that more attention should be paid to the thermodynamic efficiency of energy production and use, suggesting that it is "surely the height of thermodynamic foolishness" to use a nuclear reactor operating at thousands of degrees to run a water heater to heat bath water to 30 degrees. About a third of the energy now used in the world provides low-temperature heat, which solar collectors can already achieve in many parts of the world, Hayes claims. Moreover, if the lifetime fuels costs are properly counted in economic comparisons, he contends that solar heaters are already able to compete with conventional fuels.

Another promising renewable energy source is small hydroelectric plants which could easily be constructed to serve small villages. Even in the United States, Hayes reckons, there may be as many as 50,000 potential sites available for hydropower plants as small as half a megawatt. There is also a large and relatively untapped source of energy from the production of methane from animal wastes, ethanol from fermenting plant wastes, and low-grade heat from biomass, Hayes suggests. A start has, however, been made on some of those technologies, particularly in India and China which are operating a substantial number of methane plants, and Brazil and Australia, where fermentation plants running on sugar cane and eucalyptus wastes are already in production.

As for more sophisticated uses of solar energy, such as large-scale generation of electricity, Hayes argues that although massive solar generating plants would hold considerable environmental advantages over conventional and nuclear power stations, they would also carry some of the disadvantages of inefficiency and centralisation. "A

Recombinant DNA bill proposed

THE Carter Administration last week finally proposed legislation to control all recombinant DNA experiments in the United States, after some three weeks of discussions between the Secretary of Health, Education and Welfare (HEW) and officials from the National Institutes of Health (NIH) and the White House. Although the final version of the bill conforms closely to a draft version proposed last month by an inter-agency task force, it differs in one key respect: it would allow state and local governments to establish controls on the research which are more stringent than the federal regulations.

The bill was formally introduced into the Senate last week by Senator Edward Kennedy, Chairman of the Senate Health Subcommittee which will handle the legislation. Kennedy made clear, however, that he doesn't necessarily agree with every word in the bill and in a statement he outlined a few reservations with the Administration's approach.

The bill would essentially require the Secretary of HEW to establish regulations to control recombinant DNA research in both public and private institutions. He would be required to issue interim regulations within 90 days and a final version within a year, after allowing suitable time for public comments and discussion. The interim regulations, at least, would probably be the guidelines issued last year by NIH. The bill would also require that all facilities housing recombinant DNA experiments be licensed by HEW.

There is also a provision in the bill which would empower HEW to

send inspectors into laboratories the ensure that the regulations are being followed and violations could result in a fine of \$5,000 per day. Particularly serious and wilful flouting of the rules could also result in a prison term, the bill states.

As for the provision allowing state and local governments to set stricter controls than the federal regulations, many scientists are now supporting federal legislation on the grounds that uniform national regulations would be preferable to a patchwork of local controls of varying stringency. The draft version of the Administration's bill would have accomplished that by simply making the federal controls paramount, but the final version would allow the states to add extra controls if they want to. According to Administration sources, the provision was changed so as not to throttle local debate over recombinant DNA but it also bows to the political reality that a move to pre-empt state and local control would be unlikely to be passed by Congress.

The focus of legislative activity has now shifted to Senator Kennedy's Health subcommittee, which held a public hearing last week. One provision which Kennedy may add to the bill is a requirement that the National Commission for the Protection of Human Subjects of Biomedical and Behavioural Research, which has been looking into a variety of biomedical issues for the past two years, be given the task of examining the long-term implications of genetic engineering.

Colin Norman

sensible energy strategy demands more than the simple-minded substitution of sunlight for uranium," he suggests. Photovoltaic cells, which convert sunlight directly to electricity, have the advantage of providing a decentralised power source which could be used in tandem with other small-scale generators such as windmills, Hayes notes, suggesting that photovoltaics "may offer the most exciting solar electric prospect".

Unfortunately, however, they also have a major disadvantage: their cost is at present prohibitive. Nevertheless, Hayes is optimistic. He notes in his study that the cost of photovoltaic cells has dropped from \$200,000 per peak kilowatt in 1959 to about \$13,000 today. If the trend continues, and if it is helped by economies of scale, Hayes reckons that the cost may drop to as

low as \$500 per kilowatt by 1985.

If the transition to a solar era is not made during the next 50 years, Hayes argues, "the roadblocks will have been political, not technical". With massive investments already made in coal and nuclear technologies, and a large gap between future energy supply and demand generally perceived by energy planners, the political roadblocks are formidable, however. President Carter's forthcoming energy policy, which is expected to be announced next week, is likely to provide a boost to both coal and light water reactors, though it is likely to pour some cold water on longer term nuclear plans. As for solar technologies and other renewable resources, it is expected to provide nothing of the support, and certainly nothing of the magnitude which Hayes calls for. □

BRITAIN

Gloom at the top

Details of Britain's Science Budget, were disclosed last week. Chris Sherwell reports

IF THERE is despondency in Britain's science community this week, no one should be surprised. There is gloom at the top. Details of the Science Budget given last week by the Advisory Board for the Research Councils (ABRC) covered allocations for the 1977-78 financial year just begun and guidelines for the four additional years to 1981-82. The figures are much as expected (see table), but then sliding down a curve is no more comfortable for being warned of it in advance, particularly when (as now) the low point remains invisible.

Outlining the trends, the ABRC chairman, Sir Frederick Stewart, said the 1977-78 Science Budget of nearly £249 million (1977 prices) represented a cut in real terms of more than 3% on the 1976-77 budget. The government's public expenditure White Paper published in February indicated a budget of £216 million for the year, but this was at 1976 prices. Both figures included an additional £6 million to cover the increased cost of post-graduate tuition fees in 1977-78, and this too tended to make the trend less steady than it actually was.

Discussing the guidelines, Sir Frederick drew on the White Paper's own emphasis on the provisional character of figures for the years 1980-81 and 1981-82 in describing them as "very provisional"; they were intended to provide each of the councils with a broad basis for planning. The guidelines did, however, represent a continuation of the protection of the "small and medium sciences" at the expense of high energy physics, astronomy and space.

Thus the Science Research Council (SRC) faces a 1.7% contraction annually, a cut which Sir Frederick said could amount to more than 20% over a five-year period with vexed problems like 'salary creep'—the consequence of maintaining ageing research staffs on graded incremental salary scales.

On the other hand, in line with the SRC's and the ABRC's previously announced intentions, support for engineering is to grow substantially, and is in fact the only part of the budget to show a marked increase. How much this increase will prove to be by the end of the period was uncertain, Sir

Frederick said, but it "might amount to a 60% expansion". The SRC chairman, Sir Sam Edwards, stressed, however, that even a modest effort in favour of engineering would represent a large increase on the support that it received before. He was also quick to dismiss suggestions that the perceived need to support work 'relevant' to the country's industrial needs diluted the SRC's task of supporting basic research: the SRC's support for engineering, he insisted, was for basic research, just as it was in other fields.

Sir Sam described the reductions in support for big science as more serious than the figures suggested. Much of the SRC's expenditure went on international subscriptions, for example to CERN, and that meant that the effective decline in support for domestic activity might be more than 50%, not 20%. This, he said, would happen at a time when the pressure was on for Britain to participate fully rather than merely "tick over"; there were many projects which ought to be done "bigger, better and faster" than they were being done.

The position on international subscriptions clearly remains precarious. The SRC has the money to pay them this year, Sir Sam said, but if the pound collapsed again, "I don't know where we are"; the government had not given an open-ended commitment, and no bailing out could be guaranteed if that again proved necessary. As for the SRC's maintenance of a "minimum viable level of support" for big science—a concept outlined last year as the cuts really began to bite—Sir Sam confirmed that the big science community already believed that that level had been breached. The council's view, he indicated, was that it was extremely difficult to judge precisely what that level was.

Like the guidelines, the allocations of the 1977-78 budget reflect the policy of redeployment away from big science, though as usual the lion's share necessarily goes to SRC. Also given are figures for research commissioned by government departments under the Rothschild arrangements for applied research.

For the Agricultural Research Council (ARC) income from commissioned research, representing 55% of its total budget, will grow at 0.6%, but this will only contain the 'salary creep'. The Medical Research Council (MRC) faces more of a problem. As part of the general cuts in government expenditure the Department of Health has at short notice cut by 10% its commissioned research. The Natural Environment Research Council expects to have its funds maintained from the various departments (Agriculture, Industry, Energy, Environment), but anticipates cuts in the future. Against all this, Sir Frederick said that he saw no evidence that the spending departments were taking their funds elsewhere, a possibility Rothschild originally foresaw.

There is no doubt that the research councils face problems. Apart from the usual agonising difficulties of deciding priorities and, more notably, starting new projects, they agree that their main problem is nothing less than "keeping science alive" in Britain. In that they were echoing the views contained in the recent annual survey from the University Grants Committee (UGC), which with the research councils constitutes the Dual Support System for university research.

"We are deeply concerned", said the UGC, "about the deterioration of research capabilities in the universities . . . We will continue to watch the developing situation with anxiety". Those words might well have been repeated last week by the research councils for the whole of Britain's civil science effort. □

Science Budget: Allocation and Guidelines

	£ million ¹ 1977-78	% Share	% p.a. 1978-79 to 1981-82
ARC	20.4	8.2	1.9
MRC	42.3	17.0	1.6
NERC	27.2	10.9	2.5
SRC	138.0	55.4	-1.7
SSRC	14.6	5.9	1.9
British Museum (Natural History)	4.2	1.7	0.0
Royal Society	2.2	0.9	1.0
Total	248.9	100	

¹Figures are at 1977 prices. They exclude estimated receipts of £52.5 million from commissions from government departments (ARC £24.2 million; MRC £11.7 million; NERC £16.6 million)

BRITAIN

● Unsurprisingly, yet another view of the energy problem was heard at last week's 'Energy in the 80s' conference organised by the Institute of Chemical Engineers in Middlesbrough. It came from L. G. Brookes of the UK Atomic Energy Authority (UKAEA)—speaking in his personal capacity, of course—and it was aimed at undermining the 'conventional' view.

The conventional view, said Brookes, argued that the OPEC oil price rises have been a good thing, by inducing thrift and providing an incentive to develop other alternative energies, so that reduced demand and promotion of coal, nuclear power and alternative energies will close the energy gap. According to Brookes, however, price rises are not the solution to the problem: "they are themselves the problem".

His argument is simple. High energy prices have affected the economy adversely. That has hit the rate of growth of electricity demand, upon which the rate of nuclear power installation depends. That in turn has severely reduced the rate of substitution of nuclear for fossil energy. "It follows", says Brookes, "that a reduction in energy prices (or at the very least their stabilisation) [is] our number one energy policy objective".

Brookes' leap of logic is based on his view that uranium, coal and Middle East oil are the only large sources of low cost fuel, that uranium, used in breeders, is a greater source than all others together, and that nuclear power is the cheapest, safest and cleanest way of producing electricity.

Those with problems accepting nuclear power will be quick to question the thinking behind such arguments. But there will be others who will find some of Brookes' additional conclusions controversial as well. For example: high oil prices have actually lowered "the level of economic high spirits necessary for major investment in new energy sources", and her current "flirtation" with wave and solar power, which arises "to some extent from a misdiagnosis of the problem", may do harm if it is seen as an alternative to nuclear power. There is no reason to take it for granted, he adds, that the days of cheap energy are gone forever.

The Department of Energy's view is different, naturally. Last week it announced that the £1 million feasibility study of wave power it unveiled last year is to receive a boost following the encouraging developments experienced so far. Now a total of

£2.5 million will be spent by October 1978, with the extra money going mainly to larger-scale studies.

● Smokers in Britain who have felt under increasing pressure over the past couple of years will have noticed the recent period of fits and starts experienced by Britain's cigarette industry. Measures to reduce the



health hazard from smoking announced at the beginning of last month included a strengthening of the warning on packets, moves to extend public no-smoking areas and curb advertising, and the prospect of an end to high tar brands.

Close control of tobacco substitutes and additives according to guidelines from the Independent Scientific Committee on Smoking and Health (the Hunter Committee) was also sought, and at the end of the month the government gave the go-ahead to part-substitute cigarettes, the first of which may be on sale in July, subject to agreement on long term health studies. The Hunter Committee said it had decided to raise no objection to the carefully controlled use of two cellulose-based substitutes, Cytrel, made by Celanese Corp., and New Smoking Material (NSM), made jointly by ICI and Imperial Tobacco. The cigarettes will be mixed with about 25% tobacco, will carry the government health warning and will be no cheaper.

The price of cigarettes has since gone up, and the House of Commons Expenditure Committee on Preventive Medicine has recommended legislation against advertising and advocated dearer prices still, stronger health warnings and more non-smoking accommodation.

● The National Radiological Protection Board (NRPB), which last month published a review of its recent work over the three years 1974-76 (*The*

Work of the NRPB 1974-76, HMSO, £2), published a paper last week on the radiation exposure of the British population (*Fallout in rainwater and airborne dust-levels in the UK for 1975*, NRPB-R49, HMSO, 50p). Based on the analysis for fission products and plutonium isotopes of rainwater and airborne dust collected during 1975, the results show average depositions of ^{137}Cs and ^{90}Sr in rain were less than 2% of peak values recorded in 1963 following a period of heavy weapons testing. Fallout during 1975 will contribute another 1% to the total dose people will receive.

● The departure of Francis Crick from Cambridge to the Salk Institute has directed widespread attention at academic salaries and the position of those who earn money abroad. So what are the circumstances of those at the top of the ladder?

Professors and their counterparts in MRC units are paid a minimum of £8,016 in 1976-77. There is no upper limit in universities, provided each university's average salary does not exceed £9,489. The MRC's Special Appointments Grade tops out at £10,200, though higher salaries are paid in respect of special service.

Five years ago the minimum was £5,376 and the professorial average £6,528; the cost of living index has since risen by at least 75%, while salaries have risen only 45% to 50%. Though everyone in this salary range has suffered substantial setbacks in real income, university and research council employees have suffered more than most. Many have been able to supplement their income by doing short periods of work abroad. Until 1974, this income was not taxed unless brought into the country, but in the past three years those who work abroad in a wholly separate job with duties entirely performed outside the country have been allowed only a 25% tax exemption of their earnings, regardless of where the money is kept.

The recent UK Budget has liberalised the tax position for many who work overseas under tax conditions less favourable than this, but has not made any further concessions to the category which includes visiting academics. The level of taxation on the 75% taxable will depend, of course, on the total sums earned in a year, but would typically run at a level from 45% if total taxable income in the year (including UK salary) was between £7,000 and £8,000, up to 83% for over £21,000.

IN BRIEF

Windscale inquiry dates

Mr Justice Parker, who was appointed along with two assessors to conduct the inquiry into British Nuclear Fuels Ltd's plans for an oxide fuel reprocessing plant at Windscale, is to conduct an informal preliminary meeting open to the public and press to discuss the programme and procedure for the inquiry. The aim of the meeting, on 17 May in Whitehaven, Cumbria, is to allow all concerned parties to make representations on these matters ahead of the main inquiry, which opens on 14 June also in Whitehaven.

Them and us

The difference in attitude towards foreigners between the general manager of R&D at ICI and the Chief Scientist at the Department of Industry (DoI) is that the former thinks of them as 'us' and the latter as 'them'. Duncan

Davies, who made the transition from one to the other when he left ICI at the end of March, last week expressed his regrets at losing some of his former international involvement. Talking of British industry, he said that the 'entrance fee' for innovations—the initial investment to develop an idea into a marketable product—had increased by several orders of magnitude over the past twenty years. To ensure that it is recovered, better planning was needed at the initial stages of a project. Highly trained scientists and engineers, he said, should also be trained to judge the potential of an idea in the marketing as well as the scientific world before committing industry to a large investment which it might otherwise lose.

RGO exhibition

The Royal Greenwich Observatory at Herstmonceux has put together, out of

its own resources, a small exhibition on the castle and its environment and the work done by the observatory. The castle already has numerous visitors to the Isaac Newton Telescope and to the spacious grounds; they will now be able to visit the exhibition as well.

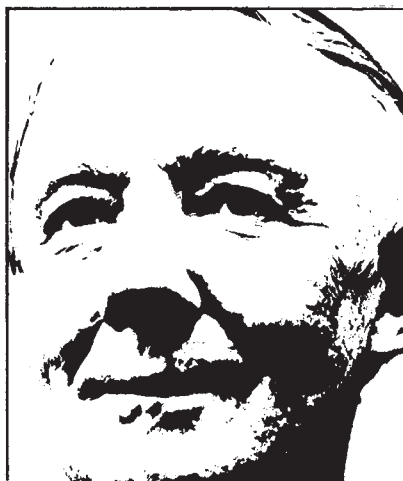
Chemical hazards report

A report published recently by the Chemical Industries Association's Safety and Health Council (CISHEC) provides the industry with details of a technique to combat potential hazards in existing and new large-scale chemical processes. The technique, known as a Hazard and Operability Study, involves systematic study of every part of the proposed process and speculative consideration of the consequences of every possible deviation from the norm. Remedial action for every potential hazard is then noted. (*A Guide to Hazard and Operability Studies*, CISHEC, £4.50.)

MANKIND has always suffered from disasters. The archetypal catastrophe was the flood, described in the Hebrew scriptures and in various middle eastern legends. Only eight individuals out of the whole human population were said to have survived to repopulate the globe with their progeny and that of the other animals saved from drowning in Noah's ark. Then in the second millennium BC the Aegean island of Santorini exploded, burying its cities in debris, and causing a tidal wave which may have ended the Minoan civilisation in Crete. This view was held by the leading Greek archaeologist, Professor Spirodon Marinatos. Shortly before his death in 1975 I heard him speak of his studies and demonstrate his excavations on Santorini. With all the signs of disaster around him he communicated his enthusiasm even when addressing a largely English-speaking audience in Greek; almost the only words I actually understood were *καταστροφή* and *κατακλυσμα*.

No one else was left to organise an expedition to help in Noah and his family's rehabilitation, and the more fortunate populations on the shores of the Mediterranean were not in a position to feed and house any survivors in Crete. Today, however, with improved communications, the rest of the world soon knows of disasters in remote corners of the globe: funds are raised and expeditions with food, medical supplies and means to improvise shelter are rapidly dispatched to try to reduce the misery of the victims. It is some-

times ironical to note that there may for generations have been chronic shortages of all the necessities, with thousands of preventable deaths each year, in these same areas, and that

Publish or perish**KENNETH MELLANBY**

world opinion has remained unmoved until a spectacular flood or earthquake has occurred. Then financial and material aid has poured in, making at least some of the population (perhaps the least deserving members) richer than ever before.

Aid for the victims of disasters has not always been appropriate in kind, nor has it always been administered efficiently. Various international agencies have built up a corpus of information, but this is not readily available to those wishing to help.

For this reason the London Technical Group has launched a new quarterly *Disasters: The International Journal of Disaster Studies and Practice*, aimed at educating scientists and relief workers. It says it will describe both successful and unsuccessful experiments in disaster relief.

The first number reports on a UNESCO conference on the assessment and mitigation of earthquake risk. There are articles on the drought in the Sahel and on emergency shelters. Various useful equipment is described. Perhaps the most interesting contribution is a letter based on experience of the Skopje earthquake in 1963 in Yugoslavia, which shows how the local population, with the materials already available, produced effective makeshift shelters long before expensive and inferior emergency houses could be shipped from abroad. This is one instance of how aid can be misused due to the ignorance of the well meaning. If future issues can keep up this standard, the journal deserves to succeed.

Our attitude to disasters, which we share with the insurance companies, has curious theological implications. A colleague, a member of his parochial church council, was active and successful in raising money to repair a mediaeval church whose roof suffered in the gales of January 1976. At the end of the year, surveying the relatively favourable balance sheet, he informed his somewhat surprised rector that everything should be satisfactory for the next year or two, "unless we suffered from another act of God".

correspondence

Oklo today

SIR,—It is obviously of great interest to trace the fate of the fission products from the natural nuclear reactor at the Oklo River, Gabon (17 March, page 206), but if parallels are to be drawn with present day reactors and radioactive wastes, then one further fact needs to be stressed, namely that today's world is seething with complex living organisms. 1.8×10^9 years ago there were, in all probability, only comparatively simple organisms.

Today's organisms have very considerable powers of dispersal so that any radioactive chemical which enters one organism may be transported a considerable distance as it travels through the food chain; and higher organisms (mammals for example) are 1,000 times more sensitive to radiation than are the simpler forms of life (such as bacteria) which might well have existed at Oklo. To argue that since we, as living organisms, survived Oklo we can also survive comparable releases of radioactivity today is dangerously misleading. Of course life will survive the next few hundred years, but the question is at what cost to the particular forms of life that we consider to be important such as our own species.

MALCOLM EDMUNDS

Biology Division,
Preston Polytechnic, UK

Scientists as administrators

SIR,—Your issue of 27 January, 1977 carries two articles which are complementary. The first is abstracted from Sir Hermann Bondi's broadcast and points out the very limited movement of scientists to administrative posts in the Civil Service and in industry in general; the second, by Kenneth Mellanby, provides the apparent paradox by emphasising that, in war-time, academics immediately turned to administrative tasks with great success. Mellanby goes on to ask why academics do not do this now.

In war, there was one clear objective—the enemy. The desired end was agreed by all, and a constant beacon to steer by. Not so now when national goals are confused and form the subject of bitter political wrangling and power-seeking. Yet, if, for example, the intensive cultivation of the Highlands of Scotland was essential for national survival, I have not the slightest doubt that the scientific ability

needed to do this would flow as readily and effectively as it did to defeat the Nazis.

Sir Hermann points out that scientists as administrators must be seen to be badly needed. Rightly or wrongly the scientist is still often made to feel a poor relation of the arts man in respect of administrative ability, though not in organisations such as Unilever and ICI whose effectiveness as innovators is remarkable.

This will not be changed simply by altering the structures of science courses, but by a positive effort to encourage scientists to move to administration. I would like to see advertisements for administrators, stating that applicants with a scientific and technological background are preferred, and would be given the appropriate training if needed. After all, professors of science and technology and the heads of departments in government scientific institutes began life as rather shy, young scientists: most are now capable administrators and few would doubt their capacity for argument—written and spoken—sharpened and tempered as it is by the ceaseless battle with well-meaning but uncomprehending, non-scientific administrators.

P. J. HEALD

Department of Biochemistry, The Todd
Centre, Glasgow

Cutting out abbreviations

SIR,—Adrian Smith's suggestion (10 February, page 492) that abbreviated titles of periodicals be replaced by full and accurate citations, will have the sympathy of authors, editors and secretaries. This proposal has already been tested in practice. The *British Medical Journal* stopped abbreviating titles in April 1969. A working party set up in 1968 by a Conference of Medical Editors' "strongly recommended all journals to follow suit: a majority of (medical) editors agreed to do so". The working party also reported that with full citations "the increase in type area is maximally 2% and usually much less". The editors of the *British Medical Journal* also reported that "the saving in editorial time is enormous".

When the effective use of public money on scientific research is increasingly coming under close scrutiny, any time spent by authors and indeed editors, themselves very often active in research, on such a mundane and unproductive task must be seriously questioned. If any economies result from the present practice, they must only be reflected in the balance sheets of publishing companies.

L. D. INCOLL

Department of Plant Sciences,
University of Leeds, UK

Stamps of scientific interest

The British Post Office marked the centenary of the Royal Institute of Chemistry by issuing a set of four multi-coloured stamps last month, each featuring a Nobel prize-winning development.

The two shown, at 8½p and 11p, commemorate Professor Sir Derek Barton's work on conformational analysis and the development of partition chromatography by Professor A. J. P. Martin and R. L. M. Synge. They depict the central part of the chemical structure of a steroid against a background of pharmaceutical products and instruments; and a graph, showing the different amounts of substances in a complex mixture separated by partition chromatography superimposed on the kind of pattern produced by the technique when used on paper.

The other two stamps, at 10p and 13p, commemorate the work of Sir Norman Haworth, which resulted in the first synthetic vitamin identical to that occurring in natural citrus fruits, and the work of Professors Sir William and Sir Lawrence Bragg, the father and son who developed crystallography.

Australia last year issued a set of four 18c stamps featuring famous Australian

scientists, three of whom ironically were born in England. Sir Thomas Laby (1880–1946) did much of his research on the possible uses of radium in combating cancer. Professor Griffith Taylor (1880–1963) joined the Commonwealth Weather Service in 1910 as a physiographer and then joined Scott's Antarctic expedition.

The other stamps depict the ornithologist John Gould (1804–81), regarded as the father of Australian bird study, and the anthropologist, Sir Baldwin Spencer (1860–1929), who studied the tribal customs of Australian aborigines.

Ian F. Finlay



news and views

Transferable drug resistance in the gonococcus

from J. R. Saunders

GONORRHOEA is currently the most prevalent human communicable bacterial disease. Despite the emergence of strains of the causative organism *Neisseria gonorrhoeae* (the gonococcus) with increasing resistance to penicillin, single high doses of this antibiotic have generally proved effective. The discovery last year of infections in Britain, the Far East and the USA which were refractory to this regimen has caused understandable alarm amongst clinicians (see for example, *Center for Disease Control Morbidity and Mortality Weekly Report* 25, 261; 1976; Percival *et al.*, *Lancet* ii, 1379; 1976; Phillips *Lancet* ii, 656; 1976), and the current policy of treating gonorrhoea 'blind' with penicillin has therefore been questioned. The culprits for these recent outbreaks are highly penicillin-resistant gonococci producing the TEM β -lactamase (penicillinase). This enzyme is specified by the widely dispersed transposon A (TnA) and its acquisition by *N. gonorrhoeae* had been widely predicted. TnA is commonly located on R plasmids in the Enterobacteriaceae, *Pseudomonas aeruginosa* and *Haemophilus influenzae* and it seems probable that gonococci have acquired it by contact with one of these organisms.

Almost all gonococci so far studied contain a plasmid of about 2.5 Mdal which has no known function (see for example Mayer *et al. Infect. Immun.* 10, 1974; Palchaudhuri *et al. Infect. Immun.* 11, 114; 1975). Some gonococcal strains contain an additional plasmid of about 25 Mdal (Stiffler *et al. J. Bact.* 122, 1293; 1975). But so far all known antibiotic resistances in *N. gonorrhoeae* have been shown to be determined by chromosomal genes. Two groups working independently, Roberts and Falkow (this issue of *Nature*, page 630) and B. I. Eisenstein *et al. (Science* 195, 998; 1977) now show that at least two species of β -lactamase plasmid exist in gonococci, one, from Far East strains, of about 4.4 Mdal, and one, from British strains, of about 3.2 Mdal, in addition to the resident cryptic plasmids. The origin of these resistance

plasmids is not yet clear. They may have been received in entirety from other Gram negative bacteria, in particular from *H. influenzae*, which contains similar β -lactamase plasmids (de Graaf *et al. J. Bact.* 126, 439; 1976). Alternatively they may have arisen by transposition of TnA to resident gonococcal plasmids. The 'donors' of TnA in this case could have been R plasmids which are capable of entering but not subsequently maintaining themselves in *N. gonorrhoeae*. Whatever mechanism is involved, the different plasmid complements of the British and Far East strains suggest that the TEM β -lactamase gene has been acquired by *N. gonorrhoeae* on at least two separate occasions. The 4.4 Mdal β -lactamase plasmid is widely distributed amongst gonococcal strains known to be different on the basis of their nutritional requirements (auxotypes). This implies that considerable interstrain transfer has occurred. It is well known that chromosomal antibiotic resistance markers can be transferred between gonococci by transformation, which is the only mechanism of genetic exchange so far reported in *N. gonorrhoeae*. However, Roberts and Falkow have found that covalently closed circular DNA of either type of β -lactamase plasmid cannot transform *N. gonorrhoeae*. This indicates that transformation plays no significant part in the dissemination between strains of the β -lactamase plasmids.

Roberts and Falkow, and Eisenstein *et al.* have now shown that the 4.4 Mdal β -lactamase plasmid can be transferred by conjugation to other gonococci, to *Neisseria flava* and to *Escherichia coli*. This plasmid is, however, too small to encode sex factor activity itself and only those strains which also carry the 25 Mdal cryptic plasmid can act as donors. The β -lactamase producing strains isolated in the UK, which all lack this larger plasmid, cannot therefore donate penicillin resistance to other bacteria. The 25 Mdal plasmid is thus apparently acting as a gonococcal sex factor and it is of interest that this plasmid has not yet been

found in recipient bacteria which have acquired the β -lactamase plasmid from donor strains. This suggests that covalent linkage between the two plasmids is not necessary for transfer but it is not yet clear whether the gonococcal sex factor is totally incapable of transferring itself to other bacteria. However, similar mobilisation of non-self-transmissible plasmids by transfer factors is well documented in the Enterobacteriaceae. It will be of great interest to determine the molecular and genetic properties of this sex factor and whether it is of gonococcal origin.

The discovery of sex factor activity in the gonococcus has serious clinical and epidemiological implications. First, it increases the probability that more strains of *N. gonorrhoeae* will acquire genes for resistance to penicillins and other drugs. It also makes more likely the transfer of resistance genes to a close relative of the gonococcus, *N. meningitidis*, the causative agent of epidemic meningitis. Meningitis is more likely to be fatal than gonorrhoea and the situation is complicated by the fact that *N. meningitidis* is carried without ill effect in the throats of many healthy individuals. Transfer of β -lactamase genes to this organism could occur either directly from *N. gonorrhoeae*, from enteric bacteria, or from *H. influenzae*, also a common inhabitant of human throats and which can cause disease. Furthermore, *H. influenzae* has in recent years acquired both the TEM β -lactamase and the ability to conjugate. In contrast, however, the gonococcal sex factor could benefit research workers by providing a means of genetic transfer in addition to transformation for analysing the genetic determinants of pathogenicity in *N. gonorrhoeae*.

There is obviously a certain irony in the possession of sexuality by *N. gonorrhoeae*. If the gonococcal sex factor determines the synthesis of sex pili, these should provide adsorption sites on donor cells for male-specific bacteriophages. The possession by gonococci of their own venereal pathogens would indeed be divine justice. □

New Uranian satellite belt

from David W. Hughes

ON 10 March at about 21.00 universal time the planet Uranus passed across the star SAO 158687 in the constellation of Libra. Reports of this occultation have caused considerable excitement because not only was the star occulted by the planet but it was also occulted by a series of much smaller satellite objects that seem to be in an equatorial belt around the planet.

The occultation was first predicted by Gordon Taylor of the Royal Greenwich Observatory (*J. Br. astr. Assoc.* **83**, 352; 1973). No useful observations of an Uranian occultation had been made before and even for this one the occultation would only be visible from a small area of the Earth. Also Uranus would be about 40 times brighter than SAO 158687 which has a photographic magnitude of +10, so Taylor predicted that the observations would be extremely difficult and would need complex photoelectric equipment.

Occultation observations are important because they can provide data as to the size of the planet, its limb darkening and the extent and composition of its atmosphere. They also provide an accurate spot measurement of the planet's position which is extremely important when it comes to using celestial mechanics to calculate planetary perturbations.

The positions of Uranus, given in the *Astronomical Ephemeris*, and of the star SAO 158687, given in the *Smithsonian catalogue*, were carefully checked in January 1977 using photographic plates obtained with the 155-cm astrometric reflector at Flagstaff, Arizona. Franz, Wasserman, Seidemann, Van Flandern and Marsden (*IAU Circ.* 3038/40) reported that the position of the star had to be corrected by +1.15 arcs in declination. Transit circle observations of Uranus indicated that its declination should also be corrected by -0.22 arcs. This meant that the occultation would not be visible everywhere that Uranus was above the horizon, but would only be seen in an area whose northern limit extended from equatorial Africa, across the Indian Ocean, somewhat north of Perth, Western Australia and then into daylight. There was an uncertainty of about $\pm 2,500$ km in this northern limit caused by the uncertainty in the positions of the planet and star and an additional uncertainty of about 1,500 km due to the fact that the exact radius of Uranus is not known. Occultation would occur near 21 h 00 min UT and the duration would increase as one moved south.

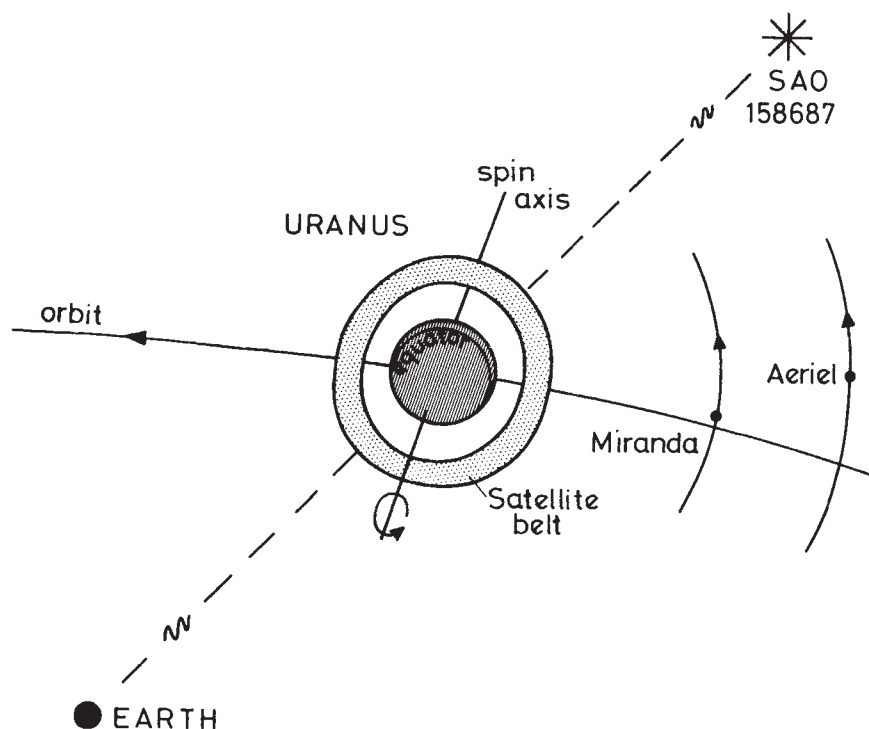


Fig. 1 A schematic diagram of Uranus and its newly discovered satellite belts at mid-occultation on 10 March, 1977. Miranda and Ariel are the two innermost satellites, SAO 158687, the occulted star, is a 10th magnitude, K5 spectral type star in Libra. The equator of Uranus, the satellite belt and the orbital planes of Ariel, Umbriel, Titania, Oberon and Miranda are all inclined at about 98° to the ecliptic plane of the Solar System.

On 10 March heavy rain prevented observations in the vicinity of Johannesburg; however astronomers on board the Gerald P. Kuiper Airborne Observatory flying across the southern Indian Ocean about 1,000 miles East of Kerguelen Island (long. 90E, lat. 50S) were more fortunate. The occultation lasted for about 25 min around 21 h 06 min UT. Dunham, Elliot and Mink, the Cornell University astronomers on the plane also, to their surprise, saw a series of secondary occultations that took place during an 8-9 min interval around 20 h 16 min UT and during a similar interval around 21 h 50 min. Bhattacharyya and Kuppuswamy (Indian Institute of Astrophysics) using the 102-cm reflector at Kavalur, Madras saw the secondary occultation start at 20 h 19 min 51 s and last for 8-9 s (they also observed that the star faded by 0.046 magnitude when it went behind the atmosphere of Uranus). Millis, Birch and Trout at the Perth Observatory, Western Australia saw the earlier of the two secondary occultations. All the groups independently concluded that these occultations were caused by satellites that are apparently part of a belt about 48,000 km from the centre of Uranus. This belt is shown schematically in Fig. 1. As Uranus travels

along its orbit the left hand portion of the belt occults the star first then the planet occultation occurs. Finally the right hand belt occults.

Brian Marsden (Smithsonian Astrophysical Observatory) has used the times of occultation to compute the following details (*IAU Circ.* 3048, 3051). The belt is circular, in the plane of Uranus' equator, about 12,000 km wide and extends between 42,200 and 54,300 km from the centre of Uranus.

The fact that the times of the individual secondary occultations are remarkably symmetrical with respect to the Uranian occultation suggests that the occulting material is confined to specific narrow rings in the belt. The Kavalur observation seems to have been caused by a 100 km sized satellite near the outer edge of the belt.

Elliot, of the Centre for Radio-physics and Space Research, Cornell University, is continuing the detailed study of the occultation records. It might also be possible to observe the belts directly, although a very large telescope will be required as the brightest satellite is expected to have a magnitude of about 19. At the present opposition of Uranus the belts are between 3.5 and 4.0 arcs to the north and south of the planet and 2.7 to 3.1 arcs to the east and west. □

Engineering nitrogen fixation

from Andrew Johnston

A conference entitled Genetic Engineering for Nitrogen Fixation, organised by Dr A. Hollaender, was held at Brookhaven National Laboratories, Upton, New York, on 13–17 March, 1977.

THE current interest in biological nitrogen fixation reflects the desire that an increased amount of the fixed N in crops should come from this source rather than from energy-expensive fertiliser. The aim of this meeting was to see whether such a goal might be achieved by genetic techniques. Most reports dealt with the status of current research in different aspects of nitrogen fixation, but attention was given to systems whose development might conceivably lead to an extension of the range of nitrogen-fixing organisms.

D. Helinski (University of California, San Diego) reviewed the methodology and uses of DNA cloning. He made the point that to date only the P-group R plasmids can be transferred to many bacteria of agronomic importance. However, their promiscuity and relatively large size make them unsuitable as cloning vehicles and he described methods by which the ability to replicate in a wide range of bacteria might be transferred to more appropriate vehicles.

Detailed mapping of nitrogen fixation (*nif*) genes has been carried out only in *Klebsiella pneumoniae*. C. Kennedy (ARC Unit of Nitrogen Fixation, Sussex) showed by transduction that *nif* genes fall into two closely linked clusters separated by a 'silent' region. Complementation tests have defined a new *nif* gene (*nifL*) which has the attributes of a controlling gene since the mutant is partially dominant and synthesises no nitrogenase components. F. Ausubel (Harvard University) reported that the *nif* cluster proximal to *hisD* could be cloned on the plasmid pMB9.

Turning to the genetics of other nitrogen-fixing bacteria A. Johnston (John Innes Institute, Norwich) demonstrated that the R plasmid R68.45 could promote chromosomal recombination in *Rhizobium* species and that stable recombinants could be formed in crosses between species. A single circular chromosome for *R. leguminosarum* was reported. In *Azotobacter vinelandii* many *nif* mutants have been characterised biochemically, but genetic analysis has been lacking. W. Brill (University of Wisconsin, Madison) reported that *nif* genes in these species could be transferred by

transformation, but in contrast to *K. pneumoniae* they were not closely linked. More surprising was the fact that *nif*⁺ transformants were obtained with DNA obtained from *Rhizobium*.

There have been several reports that plant lectins may determine the specificity of interactions between *Rhizobium* and legumes. D. Bauer (C. Kettering, Yellow Springs) showed that not all strains of *R. japonicum* bind the lectins of its host, the soybean. Those that did not could however, be induced to do so by whole soybean roots. This observation may resolve some of the discrepancies in the literature in which binding of lectins to some infective strains of *Rhizobium* has not been observed.

The symbiosis between *Azolla* and *Anabaena* has been used as a source of fixed nitrogen in Asian rice paddies for centuries. B. Rains (University of California, Davis) demonstrated that an equivalent of 90 kg ha⁻¹ yr⁻¹ may be contributed by this symbiosis. It is not possible however to cultivate the blue-green alga outside the plant so the goal of breeding for increased levels of fixation remains a distant one.

The role of *Spirillum* as a significant contributor of fixed nitrogen remains enigmatic. Although it was shown by J. Dobereiner (Embrapa, Brazil) to get into maize roots, it appears to contribute little to the nitrogen input of this plant though it may do so with some tropical grasses and wheat varieties.

The supply of energy to nitrogen-fixing systems was considered by several speakers to be a major limitation to increasing levels of fixation. R. Valentine (University of California, Davis) estimated that in the 'artificial nodule' in which NH₄⁺-excreting strains of *K. pneumoniae* were grown, about 20 molecules of ATP are required for every molecule of nitrogen fixed, although this ratio varied depending on environmental factors. Invariably H₂ is liberated during the reduction of N₂, but some of this energy loss may be recouped if a hydrogenase is present. H. Evans (Oregon State University, Corvallis) discussed the importance of this reaction in root nodules and pointed out that a particularly effective strain of *R. japonicum* has hydrogenase activity.

If *nif* genes are to be introduced into new hosts suitable vectors must first be obtained. Evidence that DNA from the crown-gall bacterium *Agrobacterium tumefaciens* does in fact integrate into plant DNA was given by E. Nester (University of Washington, Seattle).

DNA isolated from crown-gall tissue specifically hybridised with one particular fragment derived from *Sma*I digestion of the large tumour-inducing (Ti) plasmid of *A. tumefaciens*. This integrated plasmid is present in about 20 copies per plant cell and is transcribed in crown gall tissue, since RNA from this source hybridises specifically with it. These results were complemented by those of D. Schell (University of Ghent), who found that in some cases Ti plasmids contain large hairpin loops characteristic of some translocating sequences. In a strain which had lost both the ability to degrade octopene and to induce tumours one of the loops (length 40 μm) was missing.

The use of plant viruses as possible vectors was discussed by R. Meagher (University of Georgia, Athens). He showed that when the DNA of cauliflower mosaic virus was cloned in *E. coli* extra proteins were synthesised, but that none corresponded to normal virus proteins, which confirms the difficulties of obtaining accurate transcription and translation of eukaryote DNA in prokaryotes.

However, it was emphasised at the meeting that the most promising strategy for improving the economic contribution of biological nitrogen fixation to agriculture probably lies in the development of better types of already existing nitrogen fixation systems.

The conference also considered some of the legal, ethical and environmental implications of the scientific contributions. These discussions were strongly influenced by the present concern in the United States about the potential hazards of recombinant DNA research. This meant that while there was a general consensus of opinion that production of more efficient nitrogen-fixing plants and bacteria was desirable, a number of people were concerned about the possible environmental impact of such organisms even if they were formed by classical methods of gene transfer. □

Positronium spin conversion

from P. W. Atkins

A TECHNIQUE which can detect minute concentrations of triplet states of molecules in gases at normal pressures may have important applications in detecting small concentrations of some air pollutants. The technique depends on positronium spin conversion, a new development of the well-known inter-

conversion of *ortho* and *para* hydrogen.

The interconversion of *ortho* and *para* hydrogen by paramagnetic species has been known, studied and understood for some time. The effect depends on the two nuclei responding to the magnetic field of the paramagnetic molecule; in a collision one of the protons is closer to the unpaired spin than the other, and so its magnetic moment, and therefore its spin orientation, is changed by a greater amount. This relative reorientation of the two proton spins switches an *ortho* molecule (where the two nuclear spins are parallel) into a *para* molecule (where the nuclear spins are in opposition). The rate of interconversion is not fast because it depends on the interaction of the field of the paramagnetic species with the two nuclear magnetic moments, and these are very small. If the magnetic moments were of the size typical of electron magnetic moments (about 2,000 times larger than nuclear moments), or if the *ortho* and *para* molecules could combine in some way with the radical and by so doing change their spin angular momentum by sharing it with the paramagnetic species, the rate of *ortho-para* conversion would be very much greater.

In a recent letter W. Brandt and D. Spektor (*Phys. Rev. Lett.* **38**, 595; 1977) have, in fact, taken the step which leads to a rapid interconversion rate. They have done this by replacing the hydrogen molecules, with their weak nuclear moments, by positronium, and studying the rate of interconversion of *para* positronium (e^- and e^+ spins in opposition) and *ortho* positronium (e^- and e^+ spins parallel). The magnetic moments of e^- and e^+ are both of the order of a Bohr magneton, and so the (e^-, e^+) species can act as a sensitive probe for the existence of paramagnetic species in the gas phase (as well as in solids, as had been found previously). Alternatively, the positronium can attach to a paramagnetic species, and by a redistribution of spin angular momentum, switch from *ortho* and *para* while the species switches from triplet to singlet. In these ways positronium interconversion can detect free radicals and, as the authors show, respond to the presence of photo-excited triplet molecules in the gas phase.

The experiment is basically the observation of the quenching of scintillations from positronium in the presence of the impurity of interest (SO_2 or benzaldehyde for example). The positron source was a $5\mu\text{Ci } ^{22}\text{Na}$ sample at one end of a 25-cm cylinder of diameter 10 cm, with an ultraviolet source at the other. Two detectors monitored the scintillations; one was placed behind the ^{22}Na source, and the other next to the chamber wall. The

standard coincidence counting techniques were used to monitor the delayed coincidences between the 1.28 MeV γ ray from the source (which accompanies positron emission) and one of the 0.511 MeV γ rays from the annihilation of a positron by an electron. The experiment is repeated with and without ultraviolet illumination and coincidences for times greater than about 30 ns were stored. This choice of time delay eliminates positron annihilation from all states other than *ortho* positronium.

The role of the paramagnetic species (such as a photo-excited triplet state of benzaldehyde) is to convert the long-lived *ortho* positronium into *para* positronium, which annihilates rapidly. The authors examined argon, nitrogen, and air at atmospheric pressure with trace

impurities, and found that the cross sections for quenching are very large, of the order of $1.5 \times 10^{-12} \text{ cm}^2$ for benzaldehyde and $0.9 \times 10^{-12} \text{ cm}^2$ for SO_2 . These values are of the order of $10^4 \pi a_0^2$, where a_0 is the Bohr radius, and indicate interaction ranges of the order of 50 Å. They appear to increase as $\sqrt{T}$ in the range 300–400 K.

The importance of the effect is its sensitivity, on account of its large cross section, to very small concentrations of photomagnetic trace impurities in air; triplet concentrations as low as 10^{-8} can be detected at atmospheric pressures. The authors speculate that their work will have implications for research on gas pollution, in particular on H_2SO_4 formation by the photochemical oxidation of SO_2 to SO_3 in the atmosphere. □

Molecular human cytogenetics

from Susumu Ohno

One of the 6th series of annual ICN–UCLA symposia, entitled Molecular Human Cytogenetics, organised by R. S. Sparks and D. Comings, was held at Keystone, Colorado on 6–11 March, 1977.

THE meeting appropriately began with excellent reviews by D. E. Olins (Oak Ridge National Laboratory) on nucleosomes and by E. H. Davidson (CalTech) on DNA sequence organisation. A progress report by Davidson indicated that some of the short interspersed repetitive sequences, each a few hundred nucleotide pairs long, may actually represent the promoter or operator site for an adjacent unique DNA sequence. If RNA polymerase II can be modified by a cell type specific modifier subunit, the polymerase, so modified, can selectively transcribe a specific group of structural genes capped by the similar promoter sites. Thus, this particular class of repetitive sequences is at last gaining functional respectability.

Regardless of whether the functional subunit of nucleosomes is a histone dimer rather than a tetramer (R. Chalkey, University of Iowa), a nucleosome itself unquestionably consists of two histone tetramers that assure the equimolar representation by H2A, H2B, H3 and H4. 160 DNA base pairs may make one or two loops around a nucleosome. The distance between adjacent nucleosomes, however, may be variable. As a rule, it was thought to be represented by 60 exposed base pairs (Olins), but V. Allfrey

(Rockefeller University, New York) remarked that in the rat liver, there are 170 exposed DNA base pairs between nucleosomes. In view of a proposed flip flop mechanism involving histone tetramers of neighbouring nucleosomes, this uncertainty about internucleosome distance is annoying to say the least. What was in everybody's mind was the fate of nucleosomes during active transcription and replication of DNA.

With characteristically beautiful photomicrographs, S. L. McKnight (Columbia University, New York), from Oscar Miller's laboratory, seems to have shown that a stretch of DNA, being actively transcribed as evidenced by the presence of RNA polymerase molecules and growing transcripts, seems to be associated with as many nucleosomes as that representing an adjacent spacer sequence. Allfrey, however, was of the opinion that an actively transcribing ribosomal RNA gene of the slime mould, *Physarum polycephalum*, is either not associated with nucleosomes or those nucleosomes that maintain the association are highly modified, by acetylation of histones perhaps. The latter possibility was, of course, not ruled out by McKnight. With a rather indirect but clever method applied to HeLa cells, R. L. Seale (University of Colorado Medical Center, Denver) presented good evidence that, during DNA replication, one-half of a replication fork (therefore two-quarters of a replication loop) still maintains nucleosomes. The apparent omnipresence of nucleosomes forces us to modify much of our thinking as was evident in J. Cleaver's (University

of California School of Medicine, San Francisco) discussion of DNA repair enzymes.

From a string of beads, visible under the electron microscope, to a chromosome as seen under the light microscope, needs a great leap of the imagination. H1, which has a hydrophobic globular core and the very basic amino, as well as carboxyl, terminus is a good candidate for maintaining higher order helical packing of the string of beads by bridging nucleosomes at a certain interval. According to D. Cole (University of California, Berkeley) H5 (found in vertebrates with nucleated erythrocytes) as well as some of the so-called HMG proteins of I. Isenberg, can be considered as allies of H1. Furthermore, he has shown that each mammalian species generates not one, but several organ-specific species of H1. Data from sea urchin indicates that the basic histone genetic unit contains one each of the H1, H2A, H2B, H3 and H4 genes. Cole's findings and the similar finding by Isenberg (Oregon State University) for H2A indicates that the genome of higher vertebrates contains not one but several genetic units for histones that are the same with regard to the H4 gene but different with regard to H1 genes and to a lesser extent H2A genes. In this connection, an apparent paradox is noted with great interest. Using H4 message purified from HeLa cells, W. Prenskey (Tufts University, Boston) reported that the number of histone gene copies in the human genome is only of the order of tens rather than hundreds and is localised on the short arm of chromosome 7 near its centromere. By contrast not only with sea urchins and frogs but also with the cat, the human genome apparently contains fewer copies of each of the multigene families. This is true for the 5S RNA genes (found on the long arm of chromosome 1) and also of the 18S and 28S RNA genes (distributed among five pairs of acrocentrics—chromosomes 15, 16, 17, 21 and 22).

I wonder if humans owe their extraordinary longevity to the fact that they possess so few copies of these genes. It seems to me that having too many copies of a functionally ubiquitous gene is a curse rather than a blessing to somatic cells. Mutations which alter function (suppressor-type mutations of RNA genes, for example) sustained by a few copies out of hundreds or thousands are not likely to be particularly deleterious, and so may accumulate, resulting in more and more mistranslations and mistranscriptions leading finally to the general deterioration so characteristic of the ageing process. With fewer gene copies, mutated cells will be eliminated more efficiently thus delaying ageing.

Of winged beans and Wom Poms

from Robert M. May

ADMIRERS of Michael Flanders and Donald Swann will recall their hymn to the fabulous Wom Pom plant (Angel Record 35797): "You can do such a lot with a Wom Pom / You can use every part of it too . . . The flesh in the heart of a Wom Pom / Has the flavour of porterhouse steak / And the juice is a liquor / That will get you higher quicker . . . *Gaudeamus Wom Pom*, gladly we salute / *Vade mecum Wom Pom*, philanthropic fruit."

Nature's closest approximation to the Wom Pom may well be the little-known tropical legume, *Psophocarpus tetragonolobus* or winged bean. In its study of underexploited tropical plants with promising economic value (see *Nature* 265, 209; 1977), a panel of the US National Academy of Sciences under the direction of Noel Vietmeyer decided that the exceptional merits of the winged bean deserved a separate and deeper examination. The fruits of that work are set out in *The Winged Bean: A High-Protein Crop for the Tropics* (NAS, Washington DC, 1975).

Today the winged bean is grown only as a backyard crop in Papua New Guinea and Southeast Asia. These regions are, however, typical of the humid tropical zone that includes large parts of Central and South America, the Caribbean, Africa, Oceania, and West Asia, where protein deficiency is high and the plant is still unknown; not many edible crops with such high protein content will grow in these regions. Unlike the soybean (where only the seeds are used), all parts of the winged bean can be eaten: tubers, seeds, leaves, flowers, and shoots.

The immature pods are highly palatable, and can be eaten raw. The seeds are similar in composition to soybeans, averaging 34% protein (by dry weight) and 17% oil. The seeds give an edible oil, with a good proportion of polyunsaturated essential fatty

acids. The tuberous roots are slightly sweet, and contain 20% protein (by dry weight). This is spectacularly higher than the protein content in other edible roots and tubers such as cassava (1%), potatoes (2%), sweet potatoes (2%), or yams (2%).

Like other leguminous plants, the winged bean fixes atmospheric nitrogen by bacteria in nodules on the roots. Compared with other edible legumes, *P. tetragonolobus* appears to have more and larger nodules on its extensive root system, which may account for the plant's exceptional protein content. These factors are also probably responsible for the plant's ability to grow well in tropical soils poor in nitrogen, and in newly cleared soils (without the inoculation with appropriate soil bacteria demanded by many other tropical legumes). It can also be used in crop rotations to restore soil nitrogen.

Little is known about the varieties of *P. tetragonolobus*. Those currently cultivated require staking to produce high yields of pods and seeds. The NAS report acknowledges that this method of agriculture is impractical and uneconomical for mass cropping, and that not all winged bean pods mature at the same time, so that the plant is not likely to rival the soybean as a large-scale commercial product in the near future. "Its potential is as a subsistence crop or as a cash crop for small markets. For these purposes, the fact that it produces food during many months is an advantage, and staking is not a serious handicap".

One of the long term benefits that may well come from research on *in vitro* recombinant DNA is better crops, genetically engineered to our requirements. In the short run, however, we could make better use of what nature has provided, particularly in the tropics.

How a chromosome or rather a chromatid manages to pack 10^8 or more DNA base pairs into itself is a mystery still unresolved. After listening to discussions of sister chromatid exchanges by S. Latt (Children's Hospital Medical Center, Boston) and S. Wolff (University of California Medical Center) and of meiotic segregation by M. Moses (Duke University School of Medicine), one cannot help but marvel at the ease with which two sister DNA strands manage to sort themselves out without too much unwinding. All the tortuous models proposed so far are likely to be well off target. To discuss the mechanism of Q

and G-banding and of R-banding without knowing the packing method the chromosome uses is likely to be futile. Although D. Comings (City of Hope Medical Center) pointed out that a 5% difference in GC/AT ratio may make a 50% difference in fluorescence intensity, the fact that anti-single base antibodies (O. J. Miller) as well as G-C specific antibodies (J. H. Van de Sande), can also band chromosomes, seems to indicate that what matters is not the base composition of a whole long stretch but short stretches here and there that occupy exposed or accessible positions on the chromosome. I have amused myself with the

thought that a solitary human chromosome left in man-mouse somatic hybrids still exhibits its own characteristic banding pattern, although its chromosomal proteins by then are likely to be entirely of mouse origin.

Because of strenuous afternoon exercise on skis and skates and social evenings not many people attended the last session, on evolution, which I thought the best. Of man's relation to the great apes, both A. Wilson (University of California, Berkeley) and P. Pearson (University of Leiden) pointed out that the genetic distance between the chimpanzee and the gorilla is considerably greater than that between man and the chimpanzee. The tendency of certain autosomes to be extremely conserved was noted both in primates (Pearson) and Equidae (O. A. Ryder). Even rhesus monkeys seem to maintain a homologue of human chromosome 1. Whether such an apparent chromosomal conservation correlates well with the conservation of its individual genes, as is the case with the mammalian X, is an evolutionary question of utmost interest. As no functional significance can be assigned to highly repetitive DNA base sequences (so-called satellite DNA), their evolutionary role cannot be conjectured. Nevertheless, they have a peculiar habit of jumping around, ignoring the pair-by-pair homology of diploid organisms, the human Y-specific repetitive sequences being an exception (L. M. Kunkel, Johns Hopkins Hospital). Human satellite DNAs 1 to 4, some of them more ancient in origin than others, have apparently been jumping around as free spirits during the course of evolution that first branched out gibbons and then the anthropoid apes and man (J. R. Gosden, Western General Hospital, Edinburgh). E. Southern pointed out some years ago that the evolutionary fate of highly repetitive sequences is to become moderately repetitive by accumulation of random mutational base changes. Unless a mechanism exists for eliminating old repetitive sequences, the genome size should continue to increase with every new addition of highly repetitive sequences. While most species have apparently invented this elimination mechanism, the kangaroo rats (*Dipodomys*) of North American deserts have not. Among this group of leaping rodents larger genome sizes are correlated with a greater amount of highly repetitive DNA (F. T. Hatch, Lawrence Livermore Laboratory).

Sex chromosomes continue to unravel their mysteries ahead of autosomes, the *in vitro* study of the X-inactivation mechanism now seems possible. Using three X-linked marker enzymes, G. Martin (National Insti-

tutes of Health) reported that mouse ovarian teratoma cells, kept in an undifferentiated state with the help of the feeder layer, express the genetic activities of both X chromosomes. As soon as they are allowed to organise and differentiate one X-chromosome is inactivated. Rapidly accumulating evidence mainly due to S. S. Wachtel has all but verified the proposed testis-organising function of the H-Y antigen (S. Ohno, City of Hope Medical Center). But the actual location of this testis-determining H-Y gene is now problematical. Although the presumptive existence of this gene in multiple copies could still salvage its Y linkage, X linkage of the H-Y gene in the normally inducible state with the Y carrying its activator gene, is becoming increasingly attractive. □

Objective authentication

from C. R. Cavanaugh

Authenticity in Art was the subject of an interdisciplinary symposium at the Van Gogh Museum, Amsterdam, on 12 March 1977. The symposium, which reflected the increasing interest in the use of objective techniques in authentication, was organised by H. L. C. Jaffé (Art Historical Institute, University of Amsterdam) and L. H. van der Tweel (Van Danzig Foundation).

THE discussion clearly showed the complementarity of techniques derived from physical and physiological science, and from modern graphical analysis. S. J. Fleming (Oxford University) described changes in the pigments used by artists over the centuries. The obvious possibility of rejecting a later forgery because it contains pigments that were not available to the original artist has been extended by methods that in some cases enable the dating of different samples of the same pigment on the basis of their radioisotope ratios or trace contaminants. This has proven especially useful in the case of the ubiquitous pigment lead white which, depending on the era in which it was manufactured, contains varying amounts of impurities, or different crystalline structures (although the chemical composition remains the same). Fleming concluded that if museums and collectors regularly used physical dating techniques, the chance of success by forgers would become vanishingly small.

Physical methods are, however, of

little help in discriminating between artists of the same school, who tended to use similar materials. This problem was discussed by J. Bruyn (Art Historical Institute, Amsterdam) within the framework of the current exhibition in Utrecht of works by Jan van Scorel. Recent developments in infrared scanning methods make it possible to reveal the artist's sketches under all pigments other than black, and this technique has revealed several cases in which different parts of a painting were sketched by different artists. Bruyn further explained how an analysis of underpainting can aid in authenticating a painting, since members of a given school tend to follow the master's technique, whereas copyists attempt only to reproduce the appearance of the surface.

Prints and drawings present a special problem, since they offer little material for physical analysis. In this case, A. Perrig (University of Hamburg) demonstrated how an analysis of the fine structure of an artist's line can aid in identifying a drawing in cases in which the overall style is ambiguous. As an example, Perrig showed that the cross-hatching in several putative Michelangelos was done with a stroke that does not appear in any of his authenticated works, and through a series of steps that resembled a good detective mystery, traced their origin to Cellini.

Two different methods for describing an artist's style in objective terms were proposed by J. J. Denier van der Gon (University of Utrecht) and J. Storm van Leeuwen (Royal Library, The Hague). The first reported the results of recent experiments in which the velocity and position of an artist's hand were recorded while she sketched several types of line. When drawing repetitive lines for shading, velocity increased in proportion to line length, whereas when copying a line, or drawing an outline, velocity remained essentially constant, suggesting that the two types of activity are mediated by different neuromuscular control systems. If it were possible to deduce from a drawing which types of motion had been used for specific lines, it might be possible to identify copies, since these would presumably consist predominantly of constant-velocity lines. However, Denier van der Gon admitted that this would be technically difficult.

Storm van Leeuwen described his experience with a method that is already available for describing an artist's style in objective terms: the pictological method devised by van Danzig, which he used to conclude that only 33 of 116 paintings that were attributed to Frans Hals in the 1930s were actually from his hand; sub-

sequent authentication through, for example, pigment analysis has in general confirmed van Danzig's analysis. The heart of this method is the identification of at least 100 characteristics in well-authenticated works of a given artist. If a dubious painting contained 75% of these, van Danzig concluded that it was authentic. Storm van Leeuwen reported a high rate of success in applying pictological analysis to objects as diverse as book-bindings, but cautioned that the method is difficult to learn.

In the general discussion the view that connoisseurship must remain the ultimate test of a work of art was put forward. But this traditional view had clearly received a strong challenge from the more objective methods presented. □

Continental drift changes climate

from Peter J. Smith

THE annual mean temperature difference between the equator and the north pole is at present about 41 °C (equatorial 27 °C to polar -14 °C). But evidence from the fossil record makes it clear that temperatures during the Mesozoic, for example, were generally higher than they are now and that the large equator-north pole temperature drop currently observed is probably atypical of the past few hundred million years of geological time. So why has the temperature gradient increased? Why is late Cenozoic climate abnormal? One possibility is that since at least the Palaeozoic the Earth has been influenced by extraterrestrial effects such as variations in solar luminosity, as suggested by Ulrich (*Science* **190**, 619; 1975). However, Donn and Shaw (*Geol. Soc. Amer. Bull.* **88**, 390; 1977) now claim that the appeal to external processes is unnecessary and that climatic changes in the northern hemisphere during the Mesozoic-Cenozoic may be attributed entirely to changing terrestrial geography.

Their evidence comes from the application of a modified form of the thermodynamic meteorological model of Adem (*Tellus* **14**, 102; 1962 and subsequent papers), the purpose of which is to estimate the temperature of the Earth's surface and atmosphere from the radiation balance. The details are complex and can barely be summarised. Suffice it to say that the model involves such factors as the solar radiation received at the Earth's surface, the surface albedo (reflectivity), latent heat loss from the surface by evaporation,

heat of condensation in the atmosphere, cloudiness, heat storage in the oceans and atmosphere, the horizontal eddy diffusion of heat, and heat added to the atmosphere by short-wave and long-wave radiation. What emerges from a consideration of these thermal effects is an equilibrium temperature distribution; and the model can certainly be said to work insofar as it has been used successfully to describe the present climate.

The crucial element as far as Donn and Shaw are concerned, however, is the change in the Earth's albedo with time. The present albedo of the Earth as a whole is about 30%; that is, about 30% of the incident solar radiation is reflected back into space and is thus unavailable for warming the Earth's surface and atmosphere. But this average conceals large variations, for whereas the albedo of clouds is about 90%, that of ice and snow is 60-80% and that of certain land and water areas is less than 10%. Moreover, because of its transparency and mobility, water transports heat to a greater depth than does land and is thus more capable of storing heat. The amount of heat absorbed by the Earth's surface, and thus available for later warming of the atmosphere, is therefore dependent upon the relative proportions and positions of the different types of surface cover.

And the proportions and arrangement of continents and oceans in the northern hemisphere have changed considerably over the past 200 Myr because of continental drift and possibly polar wandering. The albedo and heat storage characteristics must likewise have changed. To see if such changes are sufficient to account for the observed climatic changes, Donn and Shaw have therefore used their modified Adem model to determine the surface temperature distribution for the early Triassic (200 Myr ago), the mid-Jurassic (150), the Palaeocene (65), the Oligocene (35) and the present. The point of beginning with the early Triassic is that this epoch preceded the separation of Europe, America and Africa and marked a time when much of the northern hemisphere was water. (Of course, much of the northern hemisphere was also under water during the Cretaceous, when large continental areas were submerged. This period has not been considered in order to avoid having to distinguish between the thermal effects of land and large epicritic seas.)

What the analysis shows is that during the early Triassic the temperature difference between equator and pole over most of the northern hemisphere was only about 20 °C. But as more and more land moved in from the southern

hemisphere and drifted towards the north pole over the succeeding 200 Myr, the equator-pole temperature drop rose to about 40 °C, which agrees well with the currently observed value of 41 °C. Throughout this period, however, the tropics remained at 25-30 °C. In other words, the increasing temperature gradient was brought about by a gradual decrease in polar temperature unaccompanied by any significant change in equatorial temperature.

The increasing gradient tied to a nearly constant equatorial temperature inevitably led to a decrease in the overall mean northern hemisphere temperature—in fact, a more or less uniform fall of about 3 °C throughout the 200 Myr. But temperature reductions in the higher latitudes were not only greater but also less uniform. In the 60-70°N band, for example, the annual mean temperature fell by about 5 °C during the first 165 Myr and by about the same over the remaining 35 Myr. During the same intervals the proportion of land in that latitude band rose 0-50% and 50-100% respectively, emphasising again the importance of 'continentality' in producing temperature decline. Incidentally, the model analysis shows that the mean 60-70°N temperature fell below freezing point 10-15 Myr ago, which is in good agreement with the onset of North American glaciation.

In summary, then, it would seem that continental drift is capable of explaining fully the gross changes in northern hemisphere temperature for the past 200 Myr. Whether the same can be said for the southern hemisphere, where the present equator-pole temperature drop is even higher at 77 °C, remains to be seen. Donn and Shaw promise that analysis later. □



100 year ago

BERLIN dealers in delicacies have recently received from the south, and especially from Upper Italy, immense quantities of edible birds which have been captured there in their flight northwards. Unfortunately there were not only snipe, fieldfare, and larks, or so-called "delicacies" among the birds sent, but also singing birds, that are never eaten in Germany, such as goldfinch, thrush, and nightingales. The animals were caught on their migratory flight by means of nets, or surprised during the night and indiscriminately killed. A new indication of the importance of an international law for bird protection!

From *Nature* **15**, 12 April, 522; 1877.

articles

Extended radio galaxies

W. A. Christiansen

National Radio Astronomy Observatory, Charlottesville, Virginia and University of North Carolina, Chapel Hill, North Carolina 27514

A. G. Pacholczyk

National Radio Astronomy Observatory, Charlottesville, Virginia and Steward Observatory, University of Arizona, Tucson, Arizona 85721

John S. Scott

University of Maryland, College Park and Kitt Peak National Observatory, Tucson, Arizona.

Observations of extended radio sources are interpreted in terms of a picture for radio source evolution involving multiple ejections of ram pressure confined plasmons in which instabilities lead to the formation of turbulent hot spots and to in situ particle acceleration. The hypothesis of ram pressure confined turbulent plasmons leads to a coherent theory of various radio phenomena including compact radio sources, radio tail galaxies and extended double radio sources.

ALTHOUGH the powerful extended double radio sources were among the first extragalactic radio sources to be extensively studied, a detailed understanding of their nature is still elusive. The ultimate source of the energy liberated in these sources remains a problem, and will not be dealt with here. We present a coherent picture of the observed structural, spectral and polarisation features of the extended double sources, assuming that an adequate amount of energy is released and properly directed in the central region of the parent object.

A natural model for double radio sources assumes the components of a given source to be ram pressure confined plasmons ejected from a galaxy into an external medium. More exotic models have been developed to explain the following difficulties: (1) Rayleigh–Taylor instabilities, induced in the plasmon because of deceleration, may cause disruption of the plasmon¹. (2) Large adiabatic energy losses are expected because of the expansion of the decelerating plasmon. (3) Recent observations indicate the presence of highly polarised ‘hot spots’ in some extended sources^{2–4}. If hot spots are confined by ram pressure, high densities of thermal matter ($n \sim 10^{-2} \text{ cm}^{-3}$) are required to achieve penetration to the observed distance. Polarisation observations, however, indicate that this thermal density is about an order of magnitude smaller ($n \sim 10^{-3} \text{ cm}^{-3}$) (refs 2, 3). (4) The relatively high equipartition magnetic field intensity in the hot spots implies synchrotron lifetimes on the order of 10^4 yr (refs 2, 3). This is only of the same order as the light travel time from the central source to the observed distance of the hot spot.

We argue that ram pressure confined plasmons do develop instabilities; however, the spectra and amplitude of the instabilities are severely limited by the bow shock flow. These limited instabilities lead to the formation of hot spots, the development of turbulence and *in situ* particle acceleration, so that the above listed arguments are inapplicable.

Observations of radio tail objects (and bent double struc-

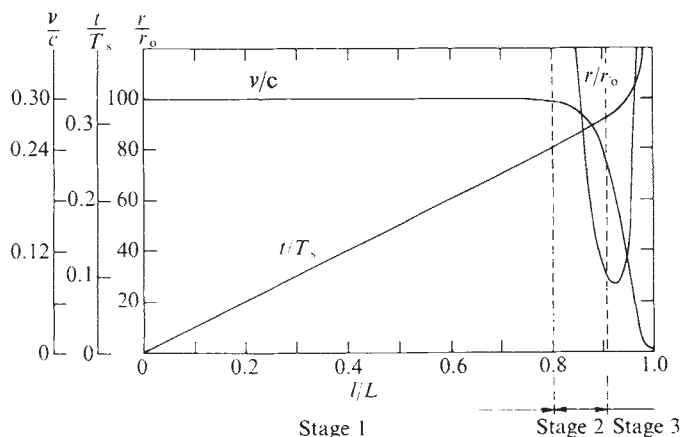
tures) which we assume to be phenomenologically similar to the more energetic doubles discussed here, clearly indicate a number of discrete ejection events (that is, multiple explosions). We therefore adopt a multiple ejection picture (compare ref. 6); this hypothesis (unlike the single ejection) leads to initial nuclear phase luminosities consistent with observations of QSOs.

Plasmons evolution

The evolution of plasmons in a multiple explosion picture for radio galaxies may be separated into four stages (Fig 1).

Stage 1: the plasmon created in the most recent explosion adiabatically expands along the channel formed by the previous explosion⁶, resulting in a low surface brightness. Stage 2: this plasmon, on encountering the debris from previous explosions, is rapidly decelerated, adiabatically compressed and greatly brightened. Stage 3: Rayleigh–Taylor instabilities develop near the front of the plasmon, and generate turbulence throughout the plasmon, leading to the acceleration of particles by the betatron and second order Fermi mechanisms^{7,8}. Stage 4: deceleration drops to zero and the plasmon expands.

Fig. 1 Velocity of the front of the plasmon in units of light velocity (V), travel time in units of stopping time t/T_s , and a scale height r/r_0 ($r_0 = 1 \text{ kpc}$) of a plasmon in a channel as functions of the position of the front of the plasmon relative to the length of the Channel l/L . Channel length: diameter is 10. The mass of the plasmon is 0.1 of the total mass of the debris at the end of the tunnel from previous events.



The instabilities no longer grow and the turbulence (and consequent particle acceleration) decays.

Instabilities and hot spots

Linearised calculations indicate that ram pressure confined plasmons are Rayleigh–Taylor and Kelvin–Helmholtz unstable. It seems that the instabilities grow rapidly enough to disrupt the plasmons¹.

Near the front of the plasmon the Rayleigh–Taylor instability is dominant with a growth time $\tau \sim (\lambda/|A|)^{1/2}$, where λ is the instability wavelength and $|A|$ is the absolute value of the plasmon acceleration. For ram pressure confined plasmons $|A| \sim \rho_B v^2 / pr$, where ρ_B is the background gas density and v , ρ and r are the centre of mass velocity, average density and scale height of the plasmon, respectively. Therefore

$$\tau \sim (\rho/\rho_B)^{1/2} (\lambda r)^{1/2} v^{-1} \quad (1)$$

As $|A|$ is largest at the beginning of Stage 3 (Fig. 1) the growth rate of all instabilities should be most rapid at this time, and the existence of hot spots and bright ridges, which we associate with growing instabilities, should be most prominently associated with this stage.

Previous calculations did not specifically take into account the interaction between growing surface instabilities and the two-dimensional hypersonic flow of shocked ambient gas around the ram pressure confined plasmon. This flow does not stabilise the surface in the sense that it prevents the growth of instabilities but it does strongly limit the amplitude of the instabilities by sweeping them around the nose of the plasmon into its wake. Therefore, these instabilities contribute to turbulence but are swept away before they reach amplitudes large enough to disrupt the plasmon.

At the stagnation point, the flow velocity of the shocked gas is zero. Above the stagnation point, however, the shocked gas flow has a non-negligible component normal to the surface¹⁰. Instabilities growing near the stagnation point encounter this flow as soon as they protrude above the surface and are efficiently suppressed.

Away from the stagnation point, the flow is nearly tangential to the surface. The tangential acceleration, A_T , of an instability due to the ram pressure of this flow is thus

$$A_T = (k \rho_{sh} v_{sh}^2) / \rho \lambda \quad (2)$$

where k is a dimensionless constant of order unity, ρ_{sh} and v_{sh} are the density and tangential velocity of the shocked gas, respectively. Numerical calculations¹⁰ indicate that

$$\rho_{sh} v_{sh} \sim \rho_B v$$

$$\text{and } v_{sh}/v \sim \sin \alpha + 2.2(h/r) \cos^3 \alpha \quad (h < 0.1r) \quad (3)$$

where α is the angle between the plasmon's velocity and the surface normal, and h is the normal distance above the surface. Instabilities continue to grow until the velocity of the instability relative to the plasmon exceeds the signal speed in the plasmon, v_s . The instability then loses communication with the rest of the plasmon, is decoupled, and ceases its growth. For ram confined plasmons, $v_s \sim v (\rho_B/\rho)^{1/2}$, and the time required for decoupling an instability is

$$t_{dc} \sim v_s/A_T = (\rho_B/\rho)^{1/2} v A_T^{-1} \quad (4)$$

Well away from the stagnation point ($\cos^3 \alpha \ll \sin \alpha \sim 1$), $A_T \sim \rho_B v^2 / \rho \lambda$. Therefore, $t_{dc} \sim (\lambda^2 \rho / v^2 \rho_B)^{1/2}$, and one may conclude that $t_{dc} < \tau$ for any $\lambda < r$. This means that Rayleigh–Taylor instabilities do not develop far from the front of a plasmon, and is consistent with observations which seem to indicate that bright hot spots (which we identify with large amplitude instabilities) are always found near the leading edges of extended sources.

Near the front of the plasmon ($\cos^3 \alpha > \sin \alpha \sim 0$), the

tangential acceleration of an instability depends on the value of h (which is equal to its amplitude). During the linear phase of instability growth

$$t_{dc} \sim (\rho/\rho_B)^{1/2} (\lambda r/hv) \quad (5)$$

where h is the average amplitude of an instability in the interval before it is decoupled. Comparing this result with the growth time (equation (1)) we conclude that instabilities near the front of the plasmon for which $(\lambda r)^{1/2} < h$ would not develop large enhancements in their amplitudes before being swept away. A natural upper limit on h is the thickness of the shocked region, $h < 0.1r$. Therefore, although the initial spectrum of instabilities is unknown, it is clear that any instability for which $(\lambda r)^{1/2} < 0.1r$ will not develop a large increase in its amplitude.

In conclusion, only a limited spectrum of Rayleigh–Taylor instabilities having wavelengths in the range

$$0.01r < \lambda < r \quad (6)$$

are capable of developing, near the front of a plasmon, the large increases in amplitude which are necessary for the enhancement of magnetic field and brightness observed in hot spots. Indeed, the sizes of observed hot spots fall in this range^{2–4}. Furthermore, there is no evidence for structure in extended radio sources at a scale smaller than the preceding lower limit⁴.

Later in Stage 3, $\rho_B/\rho \rightarrow 1.0$ and $|A| \rightarrow 0$. As a result, the instabilities stop growing, expand and blend with the extended source.

Acceleration and spectra

Equipartition magnetic fields in double-lobed sources (especially in the hot spots) lead to unacceptably large synchrotron losses in times short compared with the lifetime of the radio component. In particular, the frequency, ν_T , at which synchrotron losses become dominant (the frequency where the spectrum should begin to exhibit exponential steepening) is predicted to be within or possibly below the radio window.

This is not the case, however, for our model, in which the position of ν_T in the source's spectrum does not move steadily to lower frequencies. Because of the compression, betatron and Fermi accelerations which occur at various stages of the plasmon's evolution, ν_T at times increases.

The general form of the function $\nu_T(t)$ is¹¹

$$\nu_T = [(\text{const.})H(t) \exp(2a)] / [(\int H^2(t) \exp(a) dt)^2] \quad (7)$$

where a is the time-dependent sum of the coefficients of particle energy change (for example, Fermi acceleration coefficient, adiabatic loss coefficient, etc.). (See Pacholczyk and Scott^{7,8}.)

During Stage 1, equation (7) implies that ν_T decreases, although synchrotron losses are minimal, since the plasmon is expanded to a large scale size and the magnetic field is small.

The compression during Stage 2 implies that ν_T increases as r decreases

$$\nu_T \approx 8.2 \times 10^{23} H^{-3}(r_2) (\langle \dot{r} \rangle^2 / r_2^2) (\ln r_2/r_1)^{-2} \text{ (Hz)} \quad (8)$$

where subscripts refer to the ends of respective stages. The development of turbulence during the early parts of Stage 3 leads to a further increase in the magnetic field through the turbulent dynamo effect, implying that although r increases during Stage 3, ν_T also increases (compare equation (7)). A given component will not be perfectly homogeneous since turbulence develops at different rates in different parts of the source. This means that the frequency of the exponential cutoff, ν_T , will be a function of position in the source, with the regions where the instabilities are most highly developed (that is, hot spots) having the largest value of ν_T .

The overall spectrum early in Stage 3 represents the composite of a series of different exponential cutoffs corresponding to different parts of the plasmon. This implies that the total spectrum will begin to curve (but not exponentially cutoff) at a value of ν_T corresponding to parts of the plasmon where turbulence has not significantly developed. This value of ν_T

is that at the end of Stage 2 (compare equation (8)), which is the point in the evolutionary sequence just before the onset of turbulence. For the observed scale size (6×10^{22} cm), equipartition magnetic field (6×10^{-5} G), and $\langle \dot{r} \rangle \simeq 0.1c$ appropriate to Cyg A, we predict that the spectrum should start to bend at

$$\nu_{\text{bend}} \simeq 10^9 \text{ Hz} \quad (9)$$

a value close to that observed in this source⁶, although uncertainty in this value is relatively large.

As turbulence develops first at leading edges of plasmons, high resolution observations of sources in the early parts of Stage 3 should show less curvature (higher average ν_T) and flatter spectra at the front of the components than at the rear. There are some observational indications that this is so in Cyg A (ref. 2).

Sources well into Stage 3, with fully developed turbulence throughout the entire plasmon, will have lower overall polarisation (because of the turbulence), and ν_T will be shifted to higher frequencies through the source (see equations (7)–(9)) than younger, less polarised sources. In addition, because the plasmon is expanding during Stage 3 the integrated luminosity gradually decreases although acceleration is moving ν_T to higher frequencies so that for a given event the maximum luminosity is reached early in Stage 3.

Depolarisation and energetics

For Cyg A the diameter of the hot spots is ~ 3 kpc. The polarisation observations^{2,12} imply a cellular structure with $N = 10$ cells along the line of sight through the hot spot² and a value of $nH \sim 5 \times 10^{-7} \text{ G cm}^{-3}$, where n and H are the thermal particle density and magnetic field in the hot spot. Assuming, since the hot spot is compressed, $H = \eta H_{\text{EQ}}$ where H_{EQ} is the equipartition field for the entire plasmon, the value of the compression coefficient, η , can be determined by comparing the surface brightness of the hot spot with the total surface brightness of the plasmon

$$\eta^{n+1} = \left(\frac{F_{\text{HS}}}{F_{\text{total}}} \right) \left(\frac{r_{\text{total}}}{r_{\text{HS}}} \right)^2$$

where $F_{\text{HS(total)}}$ and $r_{\text{HS(total)}}$ indicate the observed flux and radius of the hot spot (entire plasmon), respectively, assuming $F(\nu) \sim \nu^{-\alpha}$. For Cyg A, $\eta = 3.6$; therefore $n = 2.3 \times 10^{-3} \text{ cm}^{-3}$. The identification of hot spots as instabilities invalidates the application of the ram confinement condition to the hot spots¹² themselves. This condition (properly applied to the entire plasmon) is consistent with the density derived above. Given the velocity, $v \sim 0.1c$, and the density derived from polarisation arguments above, the total kinetic energy of the plasmon is $E_{\text{KE}} \sim 2 \times 10^{61} \text{ erg}$. The ratio of the particle and field equipartition energy to the kinetic energy is then $E_{\text{EQ}}/E_{\text{KE}} = 1.3 \times 10^{-2}$. Therefore about 1% of the bulk kinetic energy of the plasmon has been converted through instabilities and turbulence into relativistic particle and field energy. The value for this ratio is consistent with the value of 2% derived previously in the plasmon model for the head–tail radio sources⁷.

Faraday rotation

The multiple explosion hypothesis predicts the existence of non-luminous relic plasmons outside the currently active radio-emitting sources. There is evidence that high-density relics may exist near Cyg A. Observations indicate that the south-following (Sf) component has a rotation measure of -1100 rad m^{-2} while the north preceding (Np) has a rotation measure of $+215 \text{ rad m}^{-2}$ (refs 2, 13). This large difference in the rotation measure between the two components can be interpreted as a result of the source being viewed along a line of sight which intersects one of the relic plasmons. If the source axis is not perpendicular to the line of sight (Fig. 2), radiation from the Sf component passes through a relic plasmon before reaching the observer while radiation from the Np component does not. The difference in the rotation measures would then be a measure

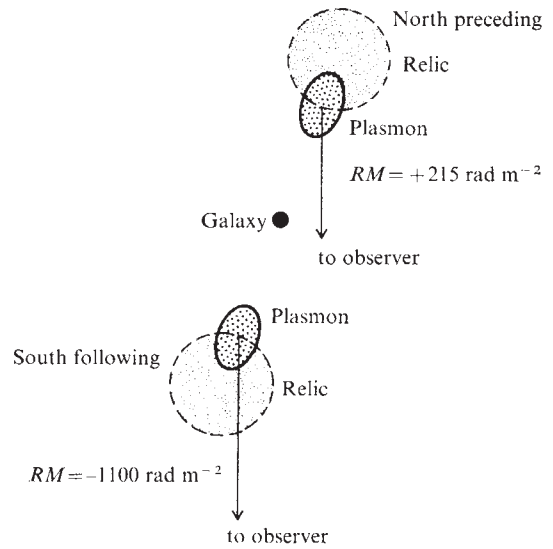


Fig. 2 Schematic representation of the radiating plasmons and relics in Cygnus A. The observed large rotation measure of the south-following component is understood in terms of the large path length of the radiation through the relic plasmon.

of the magnetic field intensity (H_{relic}), thermal particle density (n_{relic}) and diameter (d_{relic}) of the relic plasmon, that is, $H_{\text{relic}} n_{\text{relic}} d_{\text{relic}} N^{-1/2} = 10^{15} \Delta RM$, where $\Delta RM = 1315 \text{ rad}^{-2}$, and N is the number of magnetic cells along the line of sight through the relic.

The largest differences in rotation measure should occur for sources with high-density relics when the most recent plasmons are in Stage 2 or early Stage 3 and the radio axis lies near the line of sight. Later in Stage 3 when coalescence of the most recent plasmons with relics is nearly complete the rotation measure differences will disappear.

Also, for Cyg A the Sf component must be closer to the observer (Fig. 2). Therefore, because of the difference in light travel time for the emission from the two components, the Sf component should be more advanced in its evolution. If, as was assumed earlier, Cyg A is located at the beginning of Stage 3 in its evolution, the fact that the Sf component is slightly older should imply: (1) Sf luminosity $>$ Np luminosity; (2) Sf polarisation $<$ Np polarisation; and (3) the hot spot in Sf should have a higher surface brightness. All three effects are observed.

X-ray emission

Several authors have pointed out that the X-ray emission from the bow shocks of ram pressure confined plasmons will be relatively small. For Cyg A the predicted bow shock X-ray luminosity would be $\sim 10^{42} \text{ erg s}^{-1}$ (ref. 14). In addition, the inverse Compton X-ray emission from the interiors of active radio sources is not large¹⁵. The detection of an X-ray source associated with Cyg A with a luminosity of $2 \times 10^{45} \text{ erg s}^{-1}$, was reported, however, and thermal bremsstrahlung from an extended region of high temperature gas ($T \gtrsim 10^8 \text{ K}$) was suggested¹⁴.

We attribute this X-ray emission to a thermally relaxed ($T \sim 5 \times 10^8 \text{ K}$) relic plasmon whose density and mass may then be estimated

$$n_{\text{relic}} 0.51 d_{\text{relic}}^{-3/2} (\text{cm}^{-3}) \\ M_{\text{relic}} \lesssim 1.15 \times 10^{43} d_{\text{relic}}^{3/2} (\text{g}) \quad (10)$$

where d_{relic} is the diameter of the relic cloud in units of 10 kpc. Using density and rotation measure, we can estimate the relic magnetic field

$$H_{\text{relic}} \gtrsim 3 \times 10^{-8} d_{\text{relic}}^{1/2} (\text{G})$$

Assuming $n = 2 \times 10^{-3}$ (see preceding section) the mass of the currently active source in Cyg A is $M_{\text{act}} \sim 2 \times 10^{42} \text{ g}$.

Although the observed X-ray source undoubtedly is partially composed of heated intergalactic gas, a reasonable estimate of its mass would be

$$M_{\text{relic}} \sim M_{\text{act}} N_{\text{inj}} \quad (11)$$

where N_{inj} is the number of plasmons accumulated in the relic over the lifetime of the source. An upper limit on the number of explosions is

$$N_{\text{inj}} \lesssim 7 d_{\text{relic}}^{3/2} \quad (12)$$

The observed X-ray luminosity can be maintained by the injection of an energy equivalent to the currently active source in Cyg A at a rate of ~ 1 (10^8 yr^{-1}). This implies $N_{\text{inj}} \lesssim 100$, so $d_{\text{relic}} \gtrsim 60 \text{ kpc}$, $H_{\text{relic}} \gtrsim 7 \times 10^{-8} \text{ G}$, and $M_{\text{relic}} \lesssim 10^{11} M_{\odot}$.

It is interesting that with these relic parameters, the fastest process for filling the channel through which new plasmons must travel is simply back filling by the relic gas. This occurs on a time scale $\sim$ (the channel length)/(relic sound speed). For these parameters, the filling time is $\sim 10^8 \text{ yr}$, a value consistent with $N_{\text{inj}} \approx 100$, and for which the channel will remain continuously open.

Morphology

The model of radio sources presented here emphasises (1) the multiple ejection of plasmons, and (2) the *in situ* acceleration which occurs when these plasmons are significantly decelerated by ram pressure. The predicted morphology resulting from these effects should be consistent with observations.

Sources with prominent hot spots should be associated with the early parts of Stage 3. Examples other than Cyg A are 3C 33, 3C 390.3 and 3C 61.1. (The latter source also shows a strong 'bridge' feature which can be ascribed to the reflection wave generated as the plasmon 'bounces' off the relic at the beginning of Stage 3).

Other possibilities include subsequent events which occur quickly enough so that the expanded relic is still visible when the latest plasmon encounters it. An example of such a source might be DA240.

If the channel is not straight (because anisotropic ram pressure occurs when there is a density gradient in the external medium^{17,18}), some deceleration of the plasmons will occur soon after they leave the parent Galaxy, as the plasmons hit the curved wall of the channel. This causes brightening of the component close to the galaxy, resulting in such structures as bent doubles and wide-angle tails (for example, 3C465, 3C338, 1231+674, 1433+553) (ref. 19). Such structures are found in clusters of galaxies where density gradients are likely to be found.

If the velocity of ejection of the plasmons is comparable with

the speed of motion of the Galaxy through the ram-confining medium, subsequent plasmons are not ejected into the channel of the previous plasmoid. Each ejected plasmon is then decelerated by the external medium, and the resulting structure is a radio tail.

In both bent doubles and tails the deceleration process is more gradual than it would be in the case where the plasmoids encounter a massive relic. This implies that the brightness for these two cases should be smaller than for straight doubles. This effect seems to be borne out by observations¹⁹.

Because of the density gradients likely to be present, and the acceleration of the parent Galaxy, we expect straight channels to be rare in clusters of galaxies, and bright straight doubles to be rare in clusters. The exception would be cD galaxies which should lie at the centre of the density gradient and should not be significantly accelerated. Indeed, it seems bright straight doubles are rare in clusters, the exceptions being large centrally located cD galaxies.

Summary

We suggest that components of extended radio galaxies are merely plasmons, and that multiple ejections of these objects lead naturally to the observed structures of double-natured radio sources. The ram pressure deceleration of these objects (which for objects like Cyg A occurs at the end of a channel 'ploughed' by a previously ejected plasmon) leads to instabilities in the plasmon, which, far from destroying the plasmon, lead to *in situ* betatron and Fermi acceleration of the relativistic particles present in the plasmon. This acceleration provides a means of transforming a fraction of the tremendous bulk kinetic energy of the ejected plasmon into relativistic particle (and magnetic field) energy, thus producing the observed radio brightness. This replenishment of internal energy is necessary (and possibly sufficient) to explain the observed spectral and morphological features of extended radio sources.

Received 4 October 1976; accepted 18 February 1977.

- 1 Blake, G. M. *Mon. Not. R. astr. Soc.* **156**, 67 (1972).
- 2 Hargrave, P. S. & Ryle, M. *Mon. Not. R. astr. Soc.* **166**, 305 (1974).
- 3 Hargrave, P. S. & McEllin, M. *Mon. Not. R. astr. Soc.* **173**, 37 (1975).
- 4 Hargrave, P. S. & Ryle, M. *Mon. Not. R. astr. Soc.* **175**, 481 (1976).
- 5 Sheuer, P. & Jenkins, C. J. *Mon. Not. R. astr. Soc.* **174**, 327 (1976).
- 6 Christiansen, W. A. *Mon. Not. R. astr. Soc.* **164**, 211 (1973).
- 7 Pacholczyk, A. G. & Scott, J. S. *Astrophys. J.* **203**, 313 (1976).
- 8 Pacholczyk, A. G. & Scott, J. S. *Astrophys. J.* **210**, 311 (1976).
- 9 Dryer, *Cosmic Electrodynamics* **1**, 115 (1970).
- 10 Dryer, M., Rizzi, A. W. & Shen, Wen Wu, preprint (1976).
- 11 Pacholczyk, A. G. *Radio Astrophysics: Non-thermal Processes in Galactic and Extragalactic Sources* (W. H. Freeman, San Francisco, 1970).
- 12 Longair, M. S., Sheuer, P. & Ryle, M. *Mon. Not. astr. Soc.* **164**, 243 (1973).
- 13 Mitton, S. *Mon. Not. R. astr. Soc.* **153**, 133 (1971).
- 14 Longair, M. S. & Willmore, A. P. *Mon. Not. R. astr. Soc.* **168**, 479 (1974).
- 15 Okoye, S. E. *Mon. Not. R. astr. Soc.* **160**, 339 (1972).
- 16 Parker, E. N. *Astrophysics and Space Science*, **22**, 279 (1973).
- 17 Christiansen, W. A. *B.A.A.S.* **5**, 382 (1975).
- 18 Christiansen, W. A. preprint (1976).
- 19 Owen, F. N. & Rudnick, L. *Astrophys. J.* **205**, L1 (1976).

Was there a late-Würm Arctic Ice Sheet?

T. Hughes & G. H. Denton

Department of Geological Sciences and Institute for Quaternary Studies, University of Maine, Orono, Maine 04473

M. G. Grosswald

Institute of Geography, Academy of Sciences, 29 Staromonetny Street, Moscow 109017, USSR

A simplification and synthesis of late-Würm Northern Hemisphere glaciation is possible by postulating an Arctic Ice Sheet that behaved as a single dynamic system.

LATE-WÜRM Arctic glaciation is better understood if we first identify the important features of present Antarctic glaciation. The Antarctic Ice Sheet is a mass of ice and snow covering most of the Antarctic continent, with both grounded and

floating portions that are thick enough to flow under their own weight. Ice domes are centres of spreading in the grounded portions and are connected by ice divides. Terrestrial domes are grounded largely above sea level on the East Antarctic continental shield and marine domes are grounded largely below sea level on the West Antarctic continental shelf. Seaward flow from ice domes commonly converges on fast-moving currents of ice called ice streams. Ice streams draining terrestrial ice domes begin as outlet glaciers in fjords eroded through coastal mountains. Ice streams draining marine ice domes begin in submarine troughs eroded on the continental shelf. Ice streams are centres of spreading in the floating portion of an ice sheet, and form floating ice tongues that can coalesce with each other or with thick sea ice to create ice shelves; these are generally confined in embayments and pinned by islands or submarine rises. Calving bays form along the deep-water margin of the ice sheet where icebergs continuously calve from ice that is afloat or grounded well below sea level. Catastrophic retreat of a calving bay up an ice-stream occurs when the floating portion becomes unpinned and the grounded portion surges. A surge may be temporary, periodic or continuous, and is a sudden acceleration of glacial flow caused by substantial ice-bed

uncoupling, resulting in massive fracture of the ice surface.

Conventional reconstructions¹ of late-Würm glaciation emphasise the terrestrial character of the Laurentide and Scandinavian ice domes, but when isostatic depression is considered, large portions of these domes were marine, as were the Innuitian, Barents Sea, Kara Sea and East Siberian Sea domes. We have now reconstructed all of the late-Würm ice domes (CLIMAP 18,000 BP revised ice-sheet reconstructions, unpublished data, University of Maine) and find that Weertman's² idealised two-dimensional analysis of an ice dome-ice shelf junction predicts that late-Würm marine domes may have been inherently unstable for present combinations of bed slope and sea level along flowlines of major ice streams that drained these ice domes. A conclusive demonstration of whether such instability existed would require a three-dimensional analysis that considers constraints imposed on ice streams by bedrock walls and sills, and constraints imposed on ice shelves by confining embayments and pinning points²⁻⁵. A preliminary analysis⁶ indicates, however, that viscoplastic instability restricts the side-wall constraint on ice streams to a zone only a few kilometres wide, so that the major late-Würm ice streams should conform reasonably well to Weertman's two-dimensional

Fig. 1 A minimum reconstruction of the late-Würm Arctic Ice Sheet, in which an ice shelf covering the Arctic Ocean flowed from the Canadian-European sector to the Alaskan-Siberian sector, where it grounded along the continental-shelf margin and ablated slowly by melting. We assume that marine portions formed mainly from transgression of land portions on to continental shelves exposed by falling sea level. Present sea-level contour and present lacustrine shorelines — — —, Present 100-m bathymetric contour and a conservative late-Würm sea level contour, Present 1000-m bathymetric contour and, except for island arcs and ocean ridges, the approximate continental-shelf margin, ———, Limit of late-Würm glaciation. ———, Surface flowlines on the respective grounded and floating portions of the late-Würm Arctic Ice Sheet. L, Laurentide; G, Greenland; B, Barents; S, Scandinavian major domes of the Arctic Ice Sheet. IN, Innuitian; CO, Cordilleran; NE, Newfoundland; IC, Iceland; BR, British; PU, Putorana; KA, Kara; and (in Fig. 2) ES, East Siberian minor domes of the Arctic Ice Sheet., portion of the Arctic Ice Sheet that drained into the Atlantic Ocean. Regions flooded by ice-dammed rivers (black areas) include Mansi Lake in the Ob River basin, the Aral-Caspian Sea, and the Black Sea, which were 128 m above, 48 m above, and at least 50 m below present sea level, respectively, and were interconnected. +, Intersections of 10° longitude and latitude intervals.



analysis. If so, the major constraint preventing disintegration of inherently unstable late-Würm marine ice domes was probably the inertia of confined and pinned ice shelves buttressing ice streams that drained marine ice domes. This hypothesis is supported by investigations of late-Würm recession of marine West Antarctic ice from the edge of the Antarctic continental shelf²⁻⁹. Today, this dome, which occupies less than half of its late-Würm area, is drained almost entirely by large ice streams that are usually buttressed by confined and pinned ice shelves. A dynamic argument can be made that removal of the ice shelves would lead to ice-stream surges²⁻⁶, drawing down the ice dome and perhaps leading to its collapse by propagation of calving bays along evacuated ice-stream channels. Therefore, we conclude that reconstructions of late-Würm glaciers may have to consider the inertial constraint of confined and pinned ice shelves on ice streams that drained marine ice domes.

A second difficulty of standard reconstructions involves the chronology of late-Würm deglaciation. Available ¹⁴C dates indicate major retreat of peripheral terrestrial ice early in the deglacial hemicycle as opposed to relatively late major recession of northerly marine ice. For example, considerable fluctuating recession began in the southern and western terrestrial Laurentide ice shortly after 18,000 yr ago, whereas major recession of

northern Laurentide and Innuitian marine ice apparently did not begin until considerably later in the deglacial hemicycle. The reason for this is not obvious, particularly because the northern marine ice probably was unstable and because a glacial record from northern Alaska¹⁰ suggests that late-Würm climatic amelioration was nearly synchronous in this portion of the Arctic and in temperate latitudes.

In view of these problems in dynamics and deglacial chronology, we think that a simplification and synthesis of late-Würm glaciation is possible by postulating an Arctic Ice Sheet that behaved as a single dynamic system composed of terrestrial and marine grounded ice, and ice shelves (Figs 1 and 2). We start by identifying numerous ice streams in marine portions of the Arctic Ice Sheet from geological literature and from continental-shelf topography. In order to buttress the largest ice streams, we endorse the concept of an ice shelf on the Arctic Ocean¹¹⁻¹³ and postulate extensions of this ice shelf across the Greenland, Norwegian and Labrador seas into the North Atlantic. Ice-shelf flow was determined by junctions of ice shelves with domes of the Arctic Ice Sheet. Two Arctic Ice Sheet reconstructions are presented because the distribution of late-Würm Arctic ice is not well known. In both the minimum (Fig. 1) and maximum (Fig. 2) reconstructions, the Greenland and Norwegian seas

Fig. 2 A maximum reconstruction of the late-Würm Arctic Ice Sheet, assuming marine portions formed mainly from thickening sea ice which became grounded on continental shelves. Markings are described in Fig. 1 legend. Compared with the minimum reconstruction in Fig. 1, the maximum reconstruction in Fig. 2 shows a hypothetical ice dome in the East Siberian Sea as an integral part of the Arctic Ice Sheet, an expanded Innuitian ice dome, and a more southerly position of the ice-shelf calving margin in the North Atlantic. These features greatly increased the area of the Arctic Ice Sheet that drained into the Atlantic Ocean. Circumstantial evidence for the hypothetical ice dome in the East Siberian Sea included the glaciated character of the De Long Islands, the presence of foreign till and erratics on the north coast of Alaska^{14,15}; the truncated and dissected top of the Chukchi Cap¹⁶; and the topography of the sea floor in the area of Chukchi Cap, which appears glaciated and is very similar to sub-ice topography in East Antarctica (compare bathymetric contours of the Map of the Arctic Region, world 1 : 5,000,000 sheet 14, American Geographical Society, New York, 1975, with topographic contours under the East Antarctic Ice Sheet¹⁷).



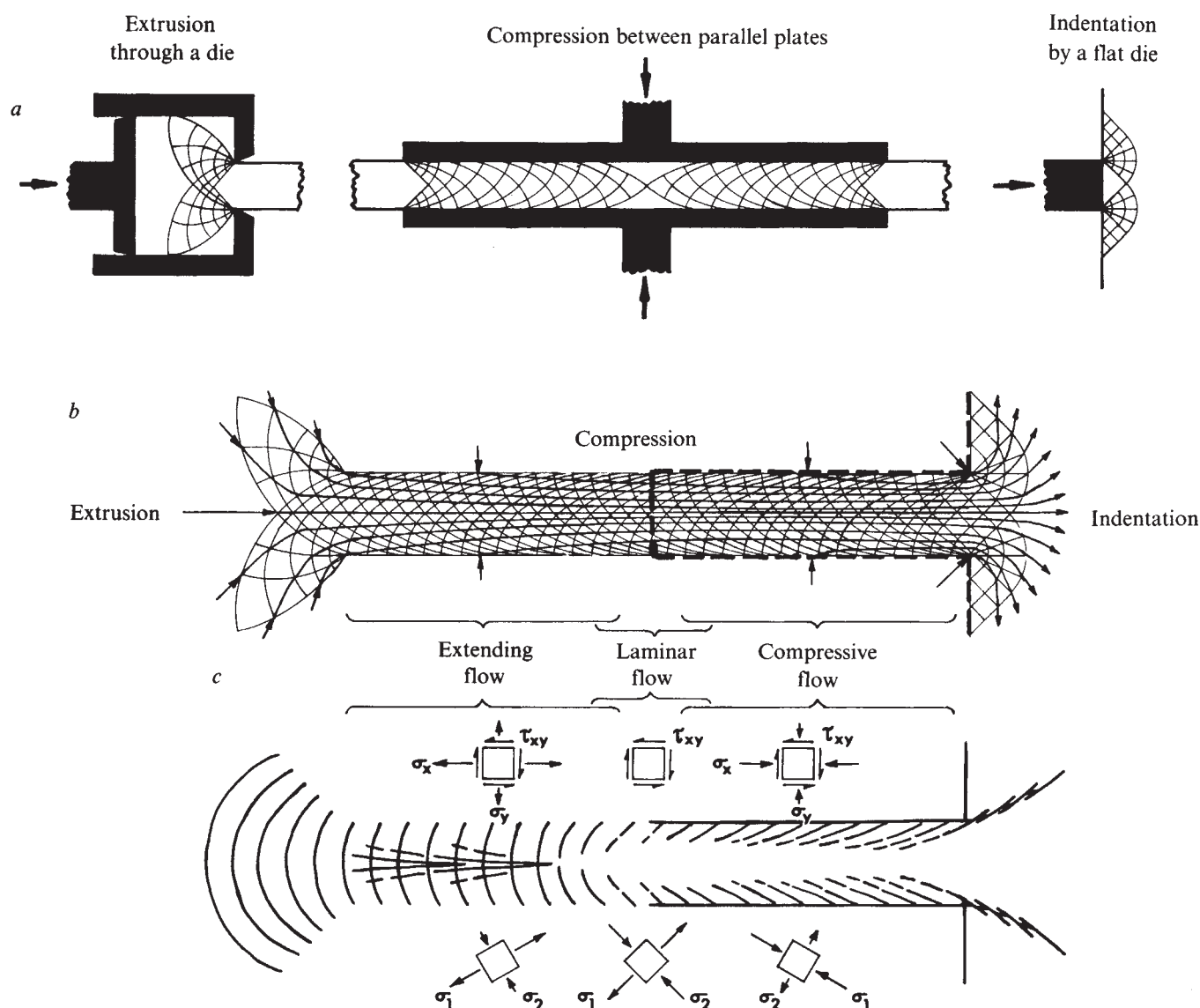


Fig. 3 The dynamics of ice streams draining marine portions of the Arctic Ice Sheet. The orthogonal slip-line fields of maximum shear stress for extrusion, compression and indentation (*a*) can be joined to give the approximate slip-line field and flow lines for an ice stream (*b*) and to predict the expected crevasse pattern (*c*) when an ice stream is the major conduit for flow from an ice dome to an ice shelf. The channel eroded by an ice stream allows the ice dome-ice shelf junction (— — —) to extend part way up the ice stream. Flowlines (→) converge on fjords through the partly subglacial mountains bordering the continental shield at the head of an ice stream, and diverge on to the ice shelf bordering the continental shelf at the foot of an ice stream. Surface components of the stress tensor σ_{13} and its principal stress components σ_1 , shown on opposite sides of the ice stream, relate crevasses to extending, laminar and compressive flow as the ice stream becomes ungrounded and merges with the ice shelf. Removal of the ice shelf would bring the extending-flow regime to the foot of the ice stream, allowing a calving bay to migrate up the ice stream as icebergs are sectioned along extending-flow crevasses. Calving-bay migration would stop when the ice-stream grounding line reached a bedrock sill above sea level at the head of the fjord through the mountains, or would proceed into the heart of a marine ice dome if a sill did not exist.

were covered by a southward-moving ice shelf that was nearly grounded on the shallow banks between Greenland, Iceland, the Faeroe Islands and Scotland; and was fed from ice domes covering these areas, Scandinavia and the Barents Sea. Both reconstructions also show a southward-moving ice shelf over Baffin Bay, David Strait and the Labrador Sea, fed from the Laurentide, Innuitian and Greenland ice domes and nearly grounded in Davis Strait. The North Atlantic margin of the combined ice shelf extended in an arc from the Grand Banks off Newfoundland to the continental shelf off Scotland, coinciding approximately with the August 0 °C sea-level isotherm 18,000 yr ago¹. This margin ablated largely by iceberg calving.

The importance of ice shelves to late-Würm glaciation is the recognition that a huge unbroken ice cover existed over polar seas and adjacent portions of North America, Greenland and Eurasia; that this ice cover constituted a unified Arctic Ice

Sheet with grounded terrestrial and marine portions and floating ice shelves; and that all components were interconnected dynamically, each depending for its existence on the others. Late-Würm glacial history can be understood by viewing the Arctic Ice Sheet as one dynamic system, stressing the predominantly marine character of this system and the dynamics of marine ice domes in relation to ice streams, ice shelves, and calving bays. The huge interior floating ice shelves were key factors in stabilising the Arctic Ice Sheet, for they buttressed marine portions which in turn abutted terrestrial portions.

Ice domes

Our proposed Arctic Ice Sheet consisted of marine domes over Hudson Bay, the Gulf of Bothnia, the Barents Sea, the Kara Sea, the Queen Elizabeth Islands and possibly the East Siberian Sea; and terrestrial domes over the Canadian Cordillera,

Newfoundland, Greenland, Iceland, the British Isles and the Putorana Plateau.

Seaward spreading from major domes of the Arctic Ice Sheet differed fundamentally from landward spreading: (1) It occurred primarily in marine portions over a bed that had been mostly below sea level, and which thus remained mostly thawed, whereas landward spreading occurred primarily in terrestrial portions over a bed that had been mostly above sea level and which had been and partly remained frozen. (2) Seaward spreading was primarily inward convergent flow from ice divides joining the domes, and thus accelerated more rapidly than landward spreading, which was primarily outward divergent flow from the ice divides. Because basal frictional heat increases as ice accelerates, more meltwater was formed at the bed for seaward than for landward spreading. (3) Seaward spreading ended as rapid stream flow because ice moved between coastal mountains and islands; landward spreading ended as slow sheet flow because ice moved over broad interior plains. Ice-sheet energy was thus focused on narrow zones along the seaward margin, but was dispersed across broad fronts along the landward margin. These features combined to increase ice-bed uncoupling, the primary cause of glacial surges, for seaward spreading compared with landward spreading. Surge conditions would thus develop first in ice streams and outlet glaciers draining marine portions of the Arctic Ice Sheet.

Ice streams

Mountain ranges along continental margins follow the landward side of the continental shelf and commonly project through an ice-dome margin. Hence, ice flowing seaward from the terrestrial portions of an ice dome commonly funnels into outlet glaciers that erode fjords through coastal mountains, and focus ice drainage in ice streams that erode troughs on the continental shelf. These ice streams are also supplied by the grounded marine portions of the ice dome. Ice-stream troughs are fore-deepened, as are fjords, so that a low submarine sill usually exists at the outer margin of the continental shelf where ice streams become afloat and merge with ice shelves. The sills and ice shelves combined to stabilise ice-stream grounding lines at many continental-shelf margins during the late-Würm. When ice-stream grounding lines retreated across these sills, they swept over the entire continental shelf and migrated into outlet-glacier fjords. We conclude that the ice streams draining the Arctic Ice Sheet were in stable equilibrium where they began as outlet glaciers, unstable equilibrium where they extended across continental shelves, and metastable equilibrium where they merged with floating ice shelves (Fig. 3). Constraints by thick ice shelves caused compressive flow, ice thickening and divergence where ice streams became afloat. This stabilised ice shelf-ice stream grounding lines at sills and bottlenecks associated with submarine banks along the outer continental-shelf margin. As soon as ice streams were able to punch through retreating ice shelves, however, the extending flow, ice thinning and convergence at the heads of ice streams became unchecked and caused grounding-line recession. The inherently unstable marine portions of the ice dome were then transformed into ice shelves and carved up by calving bays (Figs 3 and 4).

Mountains and islands along ice dome-ice shelf junctions in Antarctica are commonly unglaciated because outlet glaciers and ice streams tend to channel flow around elevated coastal topography. In our reconstruction, this implies that unglaciated portions^{18,19} of Banks Island and Baffin Island, for example, are not indicative of thin inland ice, but result from channelling of sheet flow into fjords and straits, which allowed unglaciated highlands and headlands to exist along the ice dome-ice shelf junction. Ice streams similarly drained ice around Devon Island and Newfoundland, where local ice domes may have existed.

Ice shelves

What maintained the stability of the large floating ice shelves that may have been necessary to prevent ice streams from

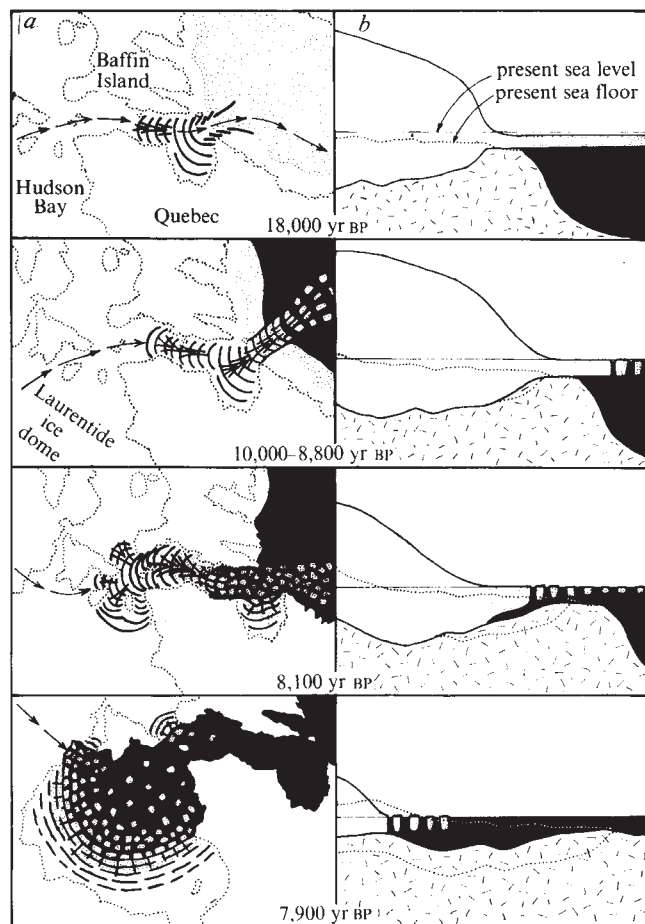


Fig. 4 Migration of a calving bay up the Hudson Strait ice stream and collapse of the Laurentide ice dome over Hudson Bay. Successive stages are shown in the plan (a) and profile (b) pairs, where the profile is taken along the ice flowline (---) shown in plan. White areas denote the grounded ice sheet, dotted areas denote the floating ice shelf and tabular icebergs, black areas denote seawater, and checked areas denote bedrock. In a: present shorelines; --- schematic crevasse patterns based on Fig. 3. The Hudson Strait ice stream was stabilised by an ice shelf in the Labrador Sea 18,000 yr ago. By about 8800–10,000 yr ago this ice shelf had retreated and thinned sufficiently for the ice stream to punch through it between the longitudinal-shear crevasses along its floating length so that a calving bay followed the ice stream grounding line up Hudson Strait. The Laurentide ice dome collapsed in a few centuries after the grounding line and calving margin of the surging ice stream crossed the island pinning points at the entrance to Hudson Bay.

drawing down marine portions of the Arctic Ice Sheet? One requirement is that a positive mass balance existed over all but calving and melting margins of the ice shelves. In the Arctic Ocean a positive annual mass balance would have been expected once northward heat transport from the North Atlantic Current essentially ceased²⁰ during the late-Würm maximum, when the North Atlantic Current flowed eastward towards Africa rather than north-eastward into the Norwegian Sea²¹. This change in current direction caused lower North Atlantic sea-surface temperatures so that they were compatible with an ice shelf that had a snowline close to sea level along its North Atlantic calving margin.

A second requirement is that snow accumulation over the Arctic Ice Sheet was sufficient to maintain the iceberg calving rate, which can be estimated from present Antarctic ice shelves, along the North Atlantic ice-shelf margin. The nearly constant forward velocity about 100 km behind the calving margin of the Ross Ice Shelf is about 0.9 km yr⁻¹ (ref. 22), and seems to

Table 1 Ice-accumulation rates on the Arctic Ice Sheet necessary to maintain a 900 m yr⁻¹ iceberg calving rate in the North Atlantic

	Minimum ice sheet (Fig. 1)	Maximum ice sheet (Fig. 2)
Hudson Strait		
Mean depth below 100 m (m)	243	243
Width over 100 m deep (km)	70	70
Area below 100 m deep (km ²)	17	17
Mean annual ice discharge (km ³ yr ⁻¹)	15	15
Area of accumulation basin (km ²)	0.55×10^6	0.55×10^6
Mean accumulation in basin (m yr ⁻¹)	0.027	0.027
Davis Strait		
Mean depth below 100 m (m)	300	300
Width over 100 m deep (km)	300	300
Area below 100 m deep (km ²)	90	90
Mean annual ice discharge (km ³ yr ⁻¹)	81	81
Area of accumulation basin (km ²)	2.36×10^6	2.36×10^6
Mean accumulation in basin (m yr ⁻¹)	0.034	0.034
Denmark Strait		
Mean depth below 100 m (m)	171	171
Width over 100 m deep (km)	350	350
Area below 100 m deep (km ²)	60	60
Mean annual ice discharge (km ³ yr ⁻¹)	54	54
Area of accumulation basin (km ²)	0.34×10^6	0.84×10^6
Mean accumulation in basin (m yr ⁻¹)	0.159	0.064
Iceland-Faeroe Ridge		
Mean depth below 100 m (m)	316	316
Width over 100 m deep (km)	380	380
Area below 100 m deep (km ²)	120	120
Mean annual ice discharge (km ³ yr ⁻¹)	108	108
Area of accumulation basin (km ²)	3.76×10^6	10.84×10^6
Mean accumulation in basin (m yr ⁻¹)	0.028	0.010
Faeroe-Shetland Trench		
Mean depth below 100 m (m)	333	333
Width over 100 m deep (km)	210	210
Area below 100 m deep (km ²)	70	70
Mean annual ice discharge (km ³ yr ⁻¹)	63	63
Area of accumulation basin (km ²)	0.72×10^6	0.72×10^6
Mean accumulation in basin (m yr ⁻¹)	0.088	0.088
North Atlantic Calving Margin		
Mean thickness 100 km from margin (m)	350	350
Width 100 km from margin (km)	1310	3800
Area 100 km from margin (km ²)	460	1330
Mean annual ice discharge (km ³ yr ⁻¹)	414	1200
Area of accumulation basin (km ²)	7.73×10^6	15.87×10^6
Mean accumulation in basin (m yr ⁻¹)	0.053	0.076

equal the iceberg calving rate because the calving margin has not undergone substantial change in this century. The nearly constant forward velocity of the Ross Ice Shelf was measured for ice averaging 350 m thick across an unsupported front 600 km wide between Roosevelt Island and Ross Island. Presumably, a forward velocity of about 0.9 km yr⁻¹ would also be required 100 km behind the calving margin of the late-Würm North Atlantic ice shelf, if its forward velocity matched the estimated present Ross Ice Shelf iceberg calving rate. Sectors between pinning points along the Labrador-Baffin-Greenland-Iceland-Faeroe-Scotland line varied from 70 to 350 km wide. Ice > 350 m thick in any sector along this line would have been grounded (Table 1). Annual precipitation rates needed to maintain a 0.9-km yr⁻¹ ice-shelf velocity across each sector are given in Table 1 (where the flowlines in Figs 1 and 2 are used to identify the snow accumulation basins for each sector) and are well below present rates in high polar regions, suggesting that the postulated calving rates could have been maintained easily. Hence, we think that late-Würm ice shelves existed over polar seas and stabilised the Arctic Ice Sheet.

Although the probable need to stabilise large ice streams that drained marine portions of the Arctic Ice Sheet is our primary reason for reconstructing ice shelves, other evidence is compatible with this postulate. Like present Antarctic ice shelves, our proposed Arctic ice shelves all existed in embayments nearly surrounded by large ice domes and had margins that were pinned by islands or banks. Glacial-marine sediments with very low abundance of left-coiling *Globigerina pachyderma* deposited in the Norwegian and Greenland seas during the late-Würm maximum indicate absence of the North Atlantic Current and presence of a complete ice cover²³. Late-Würm

sea-surface temperatures in the North Atlantic were compatible with an ice-shelf calving margin between Newfoundland and Scotland¹. Nothing yet reported from the Arctic Ocean floor precludes existence of a late-Würm ice shelf; whereas slow sedimentation rates, striated clasts and truncated rises on this ocean floor are compatible with a debris-carrying ice shelf slowly ablating on its underside and scraping across pinning points²⁴⁻²⁶.

Calving bays

We postulate that dynamic processes involving ice streams, ice shelves and calving bays were essential factors in disintegration of marine portions of the Arctic Ice Sheet. Calving bays have an important role in removing ice rapidly, and exist in metastable equilibrium. That is, they are in stable steady state for small perturbations that do not permit ice-shelf grounding lines to migrate over bedrock sills or ice-shelf calving margins to retreat from pinning points, but are in unstable catastrophic retreat for large perturbations that allow either of these events to occur. Hence, calving bays can remove floating ice shelves, or can disintegrate grounded marine ice by propagating up surging ice streams that have punched through buttressing ice shelves (Figs 3 and 4).

A view of Northern Hemisphere deglaciation that involves these dynamics is now possible. The Arctic Ice Sheet underwent peripheral fluctuations between about 22,000 and 14,000 yr ago. It then suffered step-by-step collapse of its marine portions concurrently with slow ablation of its terrestrial fringes. First, rising air temperatures and northward migration of the North Atlantic Current ablated the North Atlantic ice-shelf calving

margin from pinning points between Iceland and Scotland shortly before 14,000 yr ago²¹, causing ice-shelf recession into the Norwegian Sea. Late-Würm Scandinavian ice had extended to the edge of the Norwegian continental shelf, where it terminated in water that was commonly up to 450 m deep^{27,28}. The topography of the Norwegian continental shelf^{27,28} indicates to us that outlet glaciers from the Scandinavian dome continued across the continental shelf as ice streams up to 250 km long. If so, we would argue from the viewpoint of ice stream-ice shelf dynamics that the Norwegian Sea ice shelf buttressed these ice streams, and ice streams from the Norwegian Trench, the Barents Sea dome, and the Icelandic dome. In this case, the broad Egga moraines would have been deposited at the ice dome-ice shelf junction. Ice-shelf recession into the Norwegian Sea about 14,000 yr ago would have unbuttressed the ice streams, causing surges and resulting in rapid removal of marine ice from adjacent continental shelves by calving bays. This is compatible with the distribution and age of the oldest late-Würm moraines on the outer Norwegian and Icelandic coasts²⁹⁻³². Whether the Barents Sea dome receded at this time is unclear. Shell ¹⁴C dates from emerged marine deposits imply that major recession of Barents Sea ice occurred relatively late³³, which is compatible with ¹⁴C dates of the Marchida moraines in north-western USSR that place final disintegration of eastern Barents Sea ice in Pre-Boreal time³⁴. New dates on shells from the westernmost coast of west Spitsbergen, however, if they are reliable, suggest that initial deglaciation of western Barents Sea ice may have been earlier than previously expected (G. Boulton, personal communication, 1975). In any case, we postulate that subsequent north-westward recession of the Norwegian Sea calving bay into the Greenland Sea would have removed grounded ice from the East Greenland continental shelf. Finally, the heart of the Scandinavian ice sheet was removed by a calving bay that migrated up the region now covered by the Baltic Sea and the Gulf of Bothnia. The Gulf of Bothnia was deglaciated by 9300-8900 yr ago^{35,36}.

Late-Würm climatic amelioration also caused the ice shelf over the Arctic Ocean to unground north of Alaska and to retreat toward Siberia and Greenland, resulting in step-by-step surges of northern Laurentide, Siberian and Innuitian ice streams. Deglaciation of northern Laurentide ice probably occurred largely by calving bay propagation up these surging ice streams. It had begun by 12,400 yr ago on north-western Victoria Island³⁷; by 10,500 yr ago in Dolphin and Union Straits, and by 10,200 yr ago in Coronation Gulf. As late as 10,000 yr ago, however, a Laurentide-Innuitian ice lobe still occupied Viscount Melville Sound. Ice recession occurred in Parry Channel between the Laurentide and Innuitian domes by 9500-9000 yr ago, allowing deglaciation of McClintock Channel and Peel Sound by 9200 yr ago³⁸⁻⁴⁰.

Recession of westernmost Innuitian ice from Prince Patrick Island had begun by 11,700 yr ago and from Melville Island by 11,300 yr ago. Deglaciation of the inner channels under the Innuitian dome occurred 10,000-9000 yr ago⁴⁰. Meanwhile, the Labrador Sea calving bay had migrated past pinning points in Davis Strait and retreated into Baffin Bay by 10,000-9000 yr ago⁴¹⁻⁴⁵, then up Hudson Strait, past the island pinning points at the north end of Hudson Bay, and gutted out the remainder of the Laurentide ice dome about 8100-7900 yr ago (Fig. 4). Finally, a calving bay migrated into Foxe Basin shortly after 7000 yr ago⁴⁶. The hearts of most major domes of the Arctic Ice Sheet were thus removed, in some cases leaving terrestrial fringes to ablate by melting. The Greenland dome still remains in existence, because it alone among the major domes was largely terrestrial and therefore immune to irreversible disintegration mechanisms affecting marine ice domes.

Conclusions

We believe that the concept of an Arctic Ice Sheet harmonises geological and glaciological problems associated with standard reconstructions. It fits the pattern of deglaciation and reconciles the problem of initial major recession of peripheral terrestrial

portions as opposed to later major retreat of interior marine portions. It conforms with the dynamic relationship of grounded marine ice to ice streams and ice shelves that we postulate for the present marine West Antarctic ice dome⁷⁻⁹. Moreover, it emphasises that the Arctic Ice Sheet consisted of marine portions in unstable equilibrium confined between land portions in stable equilibrium and ice shelves in metastable equilibrium. Perturbations of stable sectors were reversible, perturbations of unstable sectors were irreversible, and perturbations of metastable sectors were reversible if small, but irreversible if large. The critical perturbations during the last 14,000 yr were intervals of climatic warming that affected the margins of the Arctic Ice Sheet. These perturbations were reversible along terrestrial margins in stable equilibrium, but were large enough to be irreversible along ice-shelf margins in metastable equilibrium. Terrestrial portions retreated slowly across wide fronts by melting. Marine portions that had been in unstable equilibrium collapsed by rapid inroads of calving bays which migrated up surging ice streams after climatic warming removed the confined and pinned ice shelves which had prevented ice-stream surges. Cryosphere-hydrosphere interactions thus destroyed the marine heart of the Arctic Ice Sheet, whereas cryosphere-atmosphere interactions eliminated terrestrial fringes.

Implicit in our Arctic Ice Sheet hypothesis is the suggestion that ice shelves formed before marine portions of the ice sheet. Otherwise, these marine portions, being inherently unstable, might never have existed. The implications of this suggestion will be presented in a later publication.

This report resulted from research sponsored by the Academy of Sciences of the USSR; and by CLIMAP and the Office of Polar Programs of the US NSF.

Received 8 December 1975; accepted 16 February 1977.

- ¹ CLIMAP *Science* **191**, 1131 (1976).
- ² Weertman, J. *J. Glaciol.* **13**, 3 (1974).
- ³ Hughes, T. *J. Geophys. Res.* **78**, 7884 (1973).
- ⁴ Robin, G. de Q. *Nature* **253**, 168 (1975).
- ⁵ Thomas, R. H. *Nature* **259**, 180 (1976).
- ⁶ Hughes, T. *ISCAP Bull.* 4, Univ. of Maine, Orono (1975); *Rev. Geophys. Space Phys.* **15**, 1 (1977).
- ⁷ Denton, G. H. & Borns, H. W. *Ant. J. U.S.* **9**, 167 (1974).
- ⁸ Denton, G. H., Borns, H. W., Jr., Grosswald, M. G., Stuiver, M. & Nichols, R. L. *Ant. J. U.S.* **10**, 160 (1975).
- ⁹ Hughes, T. *Rev. Geophys. Space Phys.* **13**, 502 (1975).
- ¹⁰ Hamilton, T. D. & Porter, S. C. *Quaternary Res.* **5**, 471 (1975).
- ¹¹ Mercer, J. H. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **8**, 19 (1970).
- ¹² Broecker, W. S. *Science* **188**, 1116 (1975).
- ¹³ Thompson, W. *Trans. Geol. Soc. Glasgow* **8**, 322 (1888).
- ¹⁴ Leffingwell, E. de K. *U.S. Geol. Surv. Prof. Paper* **109**, 1 (1919).
- ¹⁵ MacCarthy, G. R. *Arctic* **11**, 70 (1958).
- ¹⁶ Ostenso, N. A. in *Encyclopedia of Geomorphology* (ed. Fairbridge, R.), 49-55 (Reinhold, New York).
- ¹⁷ Drewry, D. J. *Nature* **256**, 194 (1975).
- ¹⁸ Andrews, J. T., Mears, A., Miller, G. H. & Pheasant, D. R. *Nature* **239**, 147 (1972).
- ¹⁹ Craig, B. G. & Fyles, J. G. *Geol. Surv. Can. Paper* **60-10**, 1 (1960).
- ²⁰ Cray, A. P. *Arctic* **13**, 32 (1960).
- ²¹ Ruddiman, W. F. & McIntyre, A. *Quaternary Res.* **3**, 117 (1973).
- ²² Dorner, E. W., Hofmann, W. & Seufert, W. J. *Glaciol.* **8**, 67 (1969).
- ²³ Kellogg, T. B. in *Climate of the Arctic* (eds Weller, G. & Bowling, S. A.) 3-36 (University of Alaska, Fairbanks, 1975).
- ²⁴ Clark, D. L. *Geol. Soc. Am.* **82**, 3313 (1971); *ibid.* **81**, 3129 (1970).
- ²⁵ Hunkins, K., Bé, A. W. H., Opdyke, N. D. & Mathieu, G. in *The Late Cenozoic Glacial Ages* (ed. Turekian, K. K.) 215-237 (Yale University, New Haven, 1971).
- ²⁶ Schwarzscher, W. & Hunkins, K. *Geology of the Arctic* **1**, 666 (1961).
- ²⁷ Andersen, B. G. in *The Quaternary* (ed. Rankama, K.) 91-138 (Wiley-Interscience, 1965).
- ²⁸ Holtedahl, O. *Norges Geol. Unders.* **208**, Pl. 14 (1960).
- ²⁹ Martinussen, M. *Norges Geol. Unders.* **315**, 1 (1974).
- ³⁰ Andersen, B. G. *Norges Geol. Unders.* **210**, 1 (1960); *ibid.* **256**, 1 (1968).
- ³¹ Mangerud, J. *Norsk Geogr. Tidsskr.* **24**, 121 (1970).
- ³² Ashwell, I. *Geogr. Annaler* **57A**, 225 (1975).
- ³³ Schytt, V., Hoppe, G., Blake, W., Jr. & Grosswald, M. G. *Int. Ass. Scient. Hydrol. Publ.* **79**, 207 (1968).
- ³⁴ Grosswald, M. G., Lavroy, A. S. & Potapenko, L. M. *Data of Glaciol. Stud. Chronicle, Discussions, Publ.* **24** (Academy of Sciences of the USSR), 173-191 (1974).
- ³⁵ Lundqvist, J. in *The Quaternary* (ed. Rankama, K.) 139-198 (Interscience, New York, 1965).
- ³⁶ Hoppe, G. *Geographica Skrifter från Uppsala Universitets Geografiska Institutionen* **20** (1948).
- ³⁷ Craig, B. G. & Fyles, J. G. *Geol. Surv. Can. Paper* **60-10**, 1 (1960).
- ³⁸ Blake, W., Jr. *Geol. Surv. Can. Paper* **63-28**, 1 (1963).
- ³⁹ Craig, B. G. *Geol. Surv. Can. Paper* **65-20**, 1 (1965).
- ⁴⁰ Blake, W., Jr. *Can. J. Earth Sci.* **7**, 634 (1970); in *Climatic Change in Arctic Areas During the Last Ten-Thousand Years* (eds Vasari, Y., Hyvärinen, H. & Hicks, S.) 77-101 (University of Oulu, 1972).
- ⁴¹ Crane, H. R. & Griffen, J. B. *Radiocarbon* **2**, 173 (1959).
- ⁴² Blake, W., Jr. in *Climatic Change in Arctic Areas During the Last Ten-Thousand Years* (eds Vasari, Y., Hyvärinen, H. & Hicks, S.) 77-101 (University of Oulu Press, 1972).
- ⁴³ Andrews, J. T. *et al.* (in the press).
- ⁴⁴ Fillon, R. H. *Nature* **253**, 429 (1975).
- ⁴⁵ *Mar. Sci. Branch Geol. Surv. Can. Paper Ser.* Dept. of Energy, Mines and Resources (in the press).
- ⁴⁶ Blake, W., Jr. *Geol. Surv. Can. Paper* **66-26**, 1 (1966).

DNA sequences coding for the H2B histone of *Psammechinus miliaris*

M. L. Birnstiel*†, W. Schaffner*‡ & H. O. Smith†

*Institut für Molekularbiologie II der Universität Zürich Winterthurerstr. 266A, 8057 Zürich, Switzerland.

†The Johns Hopkins University, Department of Microbiology, 725 N. Wolfe Street, Baltimore, Maryland 21205.

‡MRC Laboratory of Molecular Biology, University Postgraduate Medical School, Hills Road, Cambridge, UK.

*Starting from restriction enzyme cleavage sites of known topologies the DNA sequences coding for two thirds of the H2B histone protein, together with some 3' extracistronic sequences, have been determined for the sea urchin *Psammechinus miliaris*. This unambiguously identifies this gene and further reveals its 5'–3' polarity and accurate map position in a cloned histone DNA repeat unit.*

THE coding sequences of the five histone proteins are arranged¹ in the order H4, H2B, H3, H2A and H1 in a 5.6–6 kb DNA repeat unit^{1,2} which is many hundred times repeated on the chromosomal DNA of *Psammechinus miliaris*³, the first species for which the exact gene order¹ and the direction of transcription⁴ unravelled. It now seems that an identical gene arrangement is found for *Strongylocentrotus* histone DNA^{5,6}, while unpublished work from Hogness' laboratory and our own (Jean Burckhardt) suggests that the histone genes in *Drosophila* are organised differently. Here we report the base sequence arrangement of two sections of the H2B coding sequence of the histone DNA isolated⁷ from the λ -histone phage λ h22 with essentially three aims in mind.

- (1) To test the identification and map position of the H2B coding sequence, formerly obtained by hybridisation of the putative H2B mRNA to histone DNA⁸.
- (2) To verify the polarity of the H2B gene, and hence of the gene cluster, as previously determined from a combination of resection with 5' exonuclease and hybridisation with individual mRNAs to histone DNA⁴.
- (3) To confirm the accuracy of partial denaturation mapping of genes and spacers in the electron microscope².

These studies will also enable us to gain new insights into the evolution of the H2B proteins and to assess the fidelity of sequence conservation during maintenance and amplification of histone DNA cloned in a lambda vector.

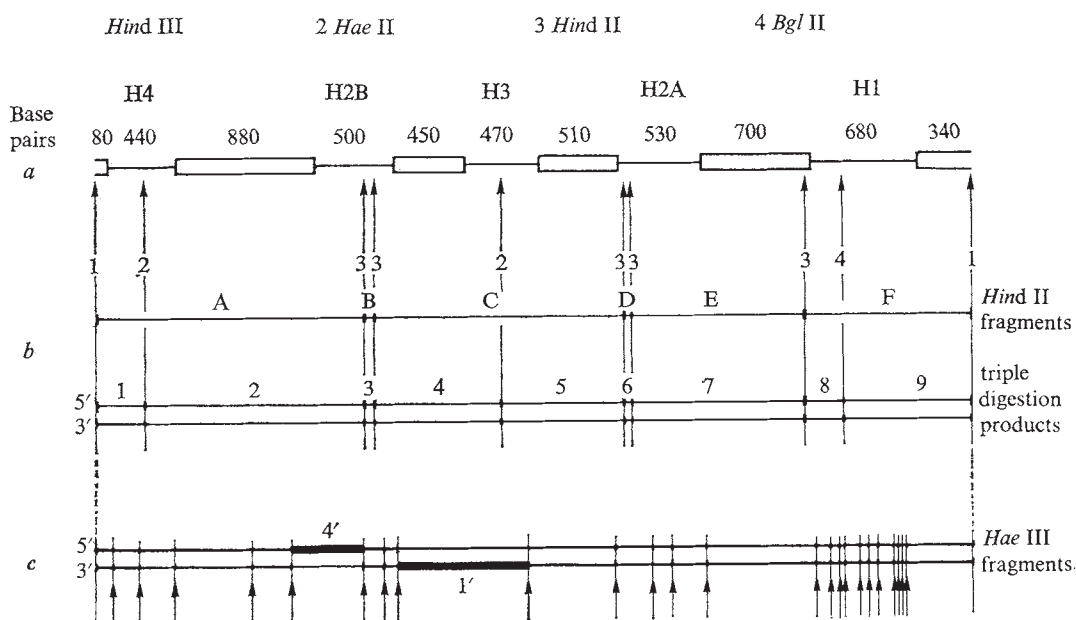
Sequencing procedures

Both the sequencing techniques^{9,10} used for these experiments depend on the availability of restriction cleavage sites as unique starting points for the actual sequence analysis.

Figure 1a depicts the structural map of the *Psammechinus* histone gene cluster as it was compiled from biochemical¹¹ and electron microscopic² analyses. The histone DNA, reclaimed from λ h22 accommodates five (possibly six; see below) cleavage sites for the restriction enzyme *Hind*II, as indicated on the map. The fragments A, C and F produced by this treatment can be further cleaved asymmetrically by digestion of the DNA with restriction enzymes *Hae*II and *Bgl*II (see Fig. 2). Two of these restriction fragments (fragments 2 and 4) terminate in close proximity to one another in the DNA segment predicted to contain the H2B sequences. The termini of these DNA fragments, with their well defined locations within the histone DNA provide suitable starting points for the sequencing of the H2B gene. Moreover, fragment B can be strand separated by gel electrophoresis⁹ and subjected to sequence analysis.

Histone DNA (25 μ g), prepared from λ h22 recombinant DNA by cleavage with *Hind*III followed by purification from λ DHA by two cycles of actinomycin CsCl density gradient centrifugation¹² was digested with 50 units *Hind*II enzyme for 3 h in 50 μ l buffer containing 20 mM Tris-HCl, pH 7.8; 7 mM MgCl₂, 7 mM mercaptoethanol. At the end of the incubation 1 μ l BAP (0.06 U) was added. Incubation was continued for 30 min at

Fig. 1 Partial denaturation and restriction maps of histone DNA reclaimed from λ h22 recombinants. *a*, Distribution of spacers (double line) and histone mRNA coding sequences as determined in the electron microscope². *b*, Cleavage sites for restriction enzymes *Hind*III, *Hae*II, *Hind*II and *Bgl*II (see text). *c*, Cleavage sites for *Hae*III. Fragments 2 and 4 and 1' and 4' were used in the sequencing experiments.



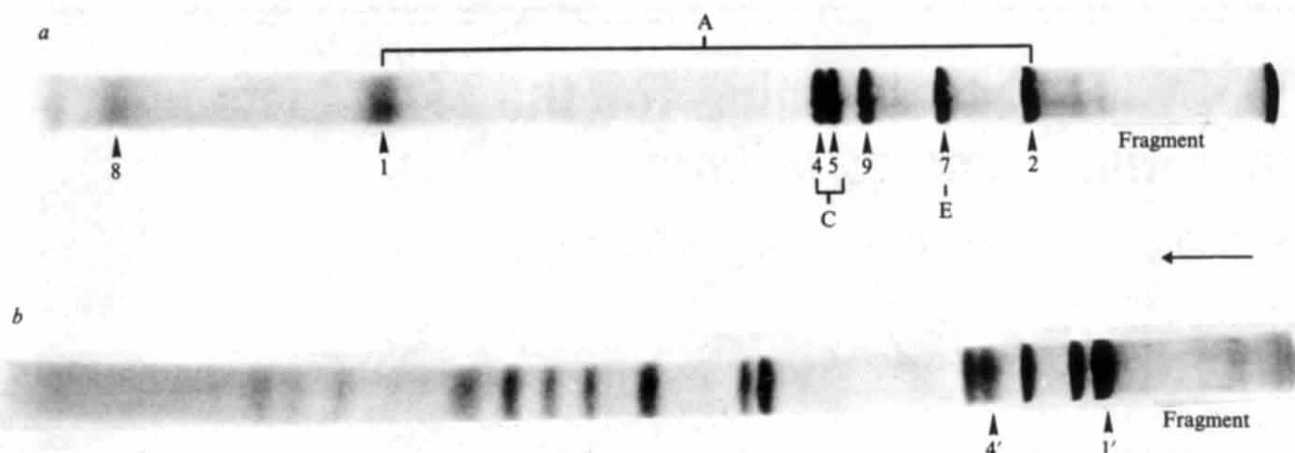


Fig. 2 Restriction fragments of cloned histone DNA h22. *a*, Electropherogramme of fragments produced after digestion of cloned histone DNA with *Hae* II, *Hind* II and *Bgl* II. Electrophoresis was through a 4% polyacrylamide gel in 90 mM Tris; 90 mM Borate, pH 8.3; 2.5 mM EDTA at 4 V cm⁻¹. The fragments refer to the DNA sections shown in Fig. 1*b*. Fragments 2 and 4, labelled at only one of their termini with (γ -³²P)-ATP were subjected to the sequencing technique of Gilbert and Maxam⁹. *b*, *Hae* III restriction fragments of cloned histone DNA. Electrophoresis was as described above. Fragments 1' and 4' were recovered and annealed to strand separated λ h22 DNA and served as primer for the 'plus and minus' technique¹⁰. For strand separation of histone recombinant phage DNA the procedure of Szybalski¹⁴ as modified by Lozeron (W. Barnes, Ph.D. thesis) was used. One mg purified λ Sam-7 h22 (ref. 7) dissolved in 3 ml of 1 mM KH₂PO₄; 1 mM EDTA; pH 8.5; 0.1% sodium sarkosyl was mixed with 0.66 mg of poly (U, G) (Miles) and was denatured for 3 min at 100 °C. After quick chilling, 60 μ l of 50 mM Tris-HCl, pH 7.6 was added and the solution made up with CsCl and water to a final vol of 16.8 ml and a density of 1.750 g ml⁻¹. The solution was transferred to a polyallomer centrifuge tube which had been previously boiled in 0.1 M EDTA, pH 10 and was centrifuged at 32,000 r.p.m. for 60 h at 20 °C in a Ti60 rotor. 40 fractions were collected, and the distribution of the separate DNA strands monitored either by absorbance measurements or by analytical agarose gel electrophoresis. Fractions corresponding to the heavy and the light strand were individually pooled and dialysed against 5 mM Tris-HCl, pH 8. Poly (U, G) was removed by incubating the samples in 0.5N NaOH at room temperature for 1 h. Tris-HCl and HCl were added to an end concentration of 0.3 M NaCl; 0.3 M Tris-HCl, pH 7.8 and the strands were self-annealed at 65 °C for 1 h. The DNA was then precipitated by addition of ethanol and redissolved in 300 μ l 10 mM Tris-HCl, pH 7.5; 0.5 mM EDTA and the solution at about 0.8 mg ml⁻¹ kept frozen. For one 'plus and minus' sequencing reaction, 10 μ g of restriction fragment (about 1 pmol) was denatured in a boiling waterbath for 3 min, chilled in ice, mixed with about 8 μ g of single stranded lambda DNA prepared as described above (about 0.5 pmol) and allowed to anneal at 67 °C for 1 h in 0.1 M NaCl; 20 mM Tris-HCl, pH 7.4; 10 mM MgCl₂; 1 mM mercaptoethanol. This material could then be used directly for elongation by DNA polymerase using the Sanger and Coulson technique¹⁰.

37 °C. The DNA was deproteinised by shaking with phenol and was recovered by precipitation with ethanol. After washing with ethanol, the DNA was redissolved in 100 μ l kinase buffer (50 mM Tris-HCl, pH 9.5; 0.01 M MgCl₂; 0.005 M DTT; 5% glycerol) to which 10 μ l of (α -³²P)-ATP (10 μ M; 1,000 Ci mmol⁻¹) made up according to the protocol of Gilbert and Maxam⁹, and 1 μ l T₄ kinase (0.5 U) were added. After 15 min 2 volumes of 2 M ammonium acetate were added and the DNA was precipitated with 21/2 volumes of ethanol. The DNA was recovered by centrifugation, dissolved in 100 μ l 10 mM Tris-HCl, pH 7.8; 1 mM EDTA and loaded on a 0.2 $\times$ 15 $\times$ 40-cm 4% polyacrylamide gel made up in glycine buffer (30 ml NaOH; 30 g glycine made up to 2 l). Electrophoresis was terminated when the unincorporated ³²P appeared in a lower buffer compartment. The labelled DNA bands were located by autoradiography and cut out from the gel.

The polyacrylamide gel revealed seven kinds of molecules rather than the five anticipated from the previous work¹¹. The two additional cleavage products were found in the low molecular weight range. One of these, B, bridges the gap between amino acid 85 and 104 of the H2B sequence and thus is made up from 62 base pairs (see below). The nature of the other small fragment, which was only poorly labelled, has not been determined as yet. The two slowest moving components of the polyacrylamide gels corresponding to fragments A and C of Fig. 1*a* were recovered, pooled and digested with *Hae* II. This treatment yielded four DNA molecules each labelled with ³²P at one of their ends. Fragments 2 and 4 (see Fig. 1*a* and 2) containing the presumptive H2B DNA sequences were then subjected to the sequencing procedure of Gilbert and Maxam⁹. In addition, fragment B was recovered, denatured and strand separated by electrophoresis and the faster moving single strand sequenced⁹.

For other sections of the histone DNA, the plus and minus method of Sanger and Coulson¹⁰ was used. This procedure is based on elongation by DNA polymerase of unique primer DNA

fragments hybridised to a single stranded template DNA. The availability of histone DNA cloned both in lambda⁷ and in pCRI plasmid¹³ proved to be very advantageous for this work. Template was obtained in preparative amounts by strand separation of histone recombinant DNA with poly(U, G) in a CsCl density gradient according to the protocol of Szybalski *et al.*¹⁴. Primer fragments were prepared by restriction of total recombinant pCRI h7 DNA and fractionation of the products by preparative polyacrylamide gel electrophoresis. This combination of template and primer DNA each obtained using different vectors eliminated any possible vector DNA background during sequencing.

For the preparation of primer DNA, 600 μ g of the recombinant plasmid pCRI (Pm histone) 7 DNA¹³ were cleaved with *Hae*III restriction endonuclease (a gift of Dr Nigel Brown) and the resulting fragments were fractionated on a 0.3 $\times$ 20 $\times$ 45 cm, 5% polyacrylamide slab gel⁹. Then 2 μ g of reclaimed 6kb *Psammechinus* histone repeat, digested with *Hae* III, were run in parallel slots in order to identify the histone-specific DNA fragments. The gel was immersed for 1 h in electrophoresis buffer containing ethidium bromide 0.5 μ g ml⁻¹. Histone DNA fragments were excised under ultraviolet light. The DNA was recovered by electrophoresis into a dialysis bag, taken up in 0.3 M sodium acetate, pH 7, mixed with 21/2 volumes of ethanol, kept at -20 °C overnight and spun down in a SW50 rotor at 45000 r.p.m. for 1 h at 4 °C. The DNA fragments were redissolved in 200 μ l 10 mM Tris-HCl, pH 7.5; 0.5 mM EDTA and kept frozen until use.

The *Hae* III fragments have been mapped (see ref. 11, and Figs 1*c* and 2). The largest of these (1') includes the spacer between the H2B and the H3 gene and part of the latter. This DNA section and 9 other *Hae* III DNA fragments were isolated (see above) and on denaturation were individually annealed to either the light or the heavy strand of λ h22 DNA known to contain the coding and the anti-coding sequence of the histone

60										65										70									
	Tyr	Lys	Val	Leu	Lys	Gln	Val	His	Pro	Asp	Thr	Gly	Ile	Ser	Ser														
5'	T A C	A A A	G T T	C T C	A A G	C A G	G T T	C A C	C C T	G A C	A C T	G G T	G T C	T C C	A G C														
3'	A T G	T T T	C A A	G A G	T T C	G T C	C A A	G T G	G G A	C T G	T G A	C C A	C A G	A G G	T C G														
	Tyr	Lys	Val	Leu	Lys	Gln	Val	His	Pro	Asp	Thr	Gly	Val*	Ser	Ser														
	Tyr	Lys	Val	Leu	Lys	Gln	Val	His	Pro	Asp	Thr	Gly	Ile	Ser	Ser														
				45					50					55															
75										80										85									
	Arg	Ala	Met	Ser	Val	Met	Asn	Ser	Phe	Val	Asn	Asp	Val	Phe	Glu														
	C G G	G C C	A T G	A C A	A T C	A T G	A A C	A G C	T T T	G T C	A A C	G A T	A T C	(T) A T C	G A G														
	G C C	C G G	T A C	T G T	T A G	T A C	T T G	T C G	A A A	C A G	T T G	C T A	T A G	(A) T A G	C T C														
	Arg*	Ala	Met	Thr*	Ile*	Met	Asn	Ser	Phe	Val	Asn	Asp	Ile*	(Ile*)	Glu														
	Lys	Ala	Met	Gly	Ile	Met	Asn	Ser	Phe	Val	Asn	Asp	Ileu	Phe	Glu														
90										95										100									
	Arg	Ile	Ala	Gly	Glu	Ala	Ser	Arg	Leu	Thr	Ser	Ala	Asn	Arg	Arg														
	A G C	A T C	G C C	G G C	G A A	G C T	T C C	C G T	C T C	A C C	C A G	T A C	A A C	A A G	A(A)														
	T C G	T A G	C G G	C C G	C T T	C G A	A G G	G C A	G A G	T G G	G T C	A T G	T T G	T T C	T(T)														
	Arg	Ile	Ala	Gly	Glu	Ala	Ser	Arg	Leu	Thr*	Gln*	Tyr	Asn	Lys	(Lys)														
	Arg	Ile	Ala	Gly	Glu	Ala	Ser	Arg	Leu	Ala	His	Tyr	Asn	Lys	Arg														
				75					80					85															
105										110										115									
	Ser	Thr	Val	Ser	Ser	Arg	Glu	Ile	Gln	Thr	Ala	Val	Arg	Leu	Leu														
	G T C	A A C	X	A T C	A G T	A G C	C G G	G A G	A T T	C A G	A C C	G C C	G T G	C G C	C T T														
	C A G	T A Y	T A G	T C A	T C G	G C C	C T C	T A A	G T C	T G G	C G G	C A C	G C G	G C G	G A A														
	(Ser)	(Thr)	Ile*	Ser	Ser	Arg	Glu	Ile	Gln	Thr	Ala	Val	Arg	Leu	Leu														
	Ser	Thr	Ile	Thr	Ser	Arg	Glu	Ile	Gln	Thr	Ala	Val	Arg	Leu	Leu														
				90					95					100															
120										125										130									
	Leu	Pro	Gly	Glu	Leu	Ala	Lys	His	Ala	Val	Ser	Glu	Gly	Thr	Lys														
	C T C	C C A	G G A	G A G	T T G	G C C	A A G	C A C	G C C	G T G	A G T	G A G	G G G	A C C	A A G														
	G A G	G G T	C C T	C T C	A A C	C G G	T T C	G T G	C G G	C A C	T C A	C T C	C C C	T G G	T T C														
	Leu	Pro	Gly	Glu	Leu	Ala	Lys	His	Ala	Val	Ser	Glu	Gly	Thr	Lys														
	Leu	Pro	Gly	Glu	Leu	Ala	Lys	His	Ala	Val	Ser	Glu	Gly	Thr	Lys														
				105					110					115															
135										140										c-term									
	Ala	Val	Thr	Lys	Tyr	Thr	Thr	Ser	Arg	c-term																			
	G C A	G T G	A C C	A A G	T A C	A C C	A C C	G T C	A A G	T A A	A C G	T T A	C A C	(T) A C T	3'														
	C G T	C A C	T G G	T T C	A T G	T G G	T G G	C A G	T T C	A T T	T G C	A A T	G T G	(A) T G A	5'														
	Ala	Val	Thr	Lys	Tyr	Thr	Thr	Val*	Lys*	ochre	untranslated region																		
	Ala	Val	Thr	Lys	Tyr	Thr	Ser	Ser	Lys	c-term																			
				120					125																				

Fig. 3 DNA sequences of H2B histone protein. Protein sequence of H2B of *Parechinus* (top line, ref. 15) of calf thymus (bottom line, ref. 16); middle line—DNA and protein sequences of the H2B of *Psammecchinus* which differ from either of the other two species are marked with asterisks. Ambiguities in either DNA or protein sequences are denoted by brackets. The restriction cleavage sites relevant to the present work are boxed.

mRNAs, respectively (see Fig. 2 legend). After base sequence analysis, the DNA sequences were converted into amino acid sequences using all three possible reading frames and compared with the known amino acid sequences of the histone proteins by computer. Sequences pertaining to the H2B were identified by comparison with the known H2B protein sequence of the sea urchin *Paracentrotus*, determined by Strickland and von Holt¹⁵.

Partial sequence of the H2B gene of *Psammechinus*

The H2B protein from the sperm of the sea urchin *Paracentrotus* contains 143 amino acids¹⁵. Comparison with the calf thymus protein revealed¹⁶ that the *Paracentrotus* sperm protein is larger by 17 amino acids, with the additional peptide sequences appearing, in the main, at the N-terminal end of the molecule. The DNA sequences obtained from the base sequence analysis of the *Psammechinus* histone DNA were converted into amino acid sequences and recorded in Fig. 3 so as to yield maximal sequence homology with the H2B protein of *Paracentrotus* (top line) and calf thymus (bottom line), respectively. The numbering of the amino acids of the *Psammechinus* protein was made to conform with that of *Paracentrotus*. Starting at the 5' terminus of fragment 2 (Fig. 1) the Gilbert and Maxam procedure yielded about 75 bases of the coding sequence, from approximately amino acid 84 to amino acid 60. Starting from the labelled *Hind*II site of fragment 4 a sequence of 80 nucleotides was obtained, representing the anti-coding sequence ranging from amino acid 107 up to 131 of the H2B protein. In this experiment, the first two or three bases were lost from the analysis so that the known sequence started at Val (position 107). The position of the *Hind*II cleavage site may be predicted from the amino acid sequence of the protein, as indicated in Fig. 3. The fast-moving single strand of B yielded the coding sequences for codons 104 to 90, leaving a gap of a few amino acids unaccounted for.

Comparison of the H2B amino acid sequences and the DNA sequence obtained from hybridisation of one of the smaller *Hae* III fragments (indicated in Fig. 1c as a bold line) to the light strand of λ h22 DNA and subsequent sequence analysis using the plus and minus method¹⁰ showed that this section contained the missing DNA sequence between amino acid positions 85 and 90. The first readable codon specified Ser in position 82 and could be extended up to amino acid 91. Here too, there was a small overlap in DNA sequence in both sets of experiments. From the position of the first readable codon 82 on the electrophoretic gel relative to the dye marker it was predicted that the *Hae* III sequence which had served as a primer for strand extension, was to be found some 18 base pairs to the left of codon 82. As can be seen from the first set of data, it lies at the predicted point between Arg 75 and Ala 76.

From the mapping experiments (Fig. 1c) it was also predicted that the large *Hae* III fragment used in these experiments encompasses most of the spacer region between the H2B and H3 gene, with a *Hae* III site just to the right of the former. Strand extension starting from the fragment annealed to the heavy λ h22 strand was expected to include the coding sequence of the C-terminal end of the H2B protein. Comparison of the DNA and protein sequences has confirmed this. The sequence contains, in addition to a short stretch of DNA, presumably coding for an untranslated region of the H2B mRNA, about 45 readable bases extending to His 127. Here again there is a small overlap of 15 bases with the previous set of data. Our data then, covering the proteins sequences from amino acid 60 to 143 provide an unequivocal identification of the H2B gene confirming our results previously obtained by the classical methods of mRNA translation *in vitro*⁸ and RNA-DNA hybridisation¹.

A small number of ambiguities in the DNA sequences and hence in the amino acid assignments remain. These have been noted in Fig. 3.

Polarity and map position of the H2B coding sequence

The positions of the *Hind*II and the *Hae* II cleavage sites have been determined independently from overlaps found in complete

digests using a variety of restriction enzymes or by our partial enzyme digestion procedure (Fig. 1). In these experiments it was established that fragment A is cleaved asymmetrically by *Hae* II so that the terminus on the left of the map becomes associated with the small DNA fragment 1 and the other terminus with fragment 2. Moving from the ³²P-labelled 5' end of fragment 2 towards the left (Fig. 1a), a partial sequence of the H2B coding sequence was obtained. In turn, the ³²P-labelled 5' terminus of fragment 4 yielded the anti-coding sequence of the H2B from amino acid 107 to 131. Also, *Hae* III fragment annealed to the light and another small fragment annealed to the heavy λ h22 DNA yielded upon extension of the 3' termini with polymerase anti-coding and coding sequences of the H2B, respectively. Together, these experiments prove unequivocally that the lower strand (Fig. 1b and c) includes the coding sequence and hence these experiments confirm that transcription of H2B is from the left to right of the map—as shown for the histone DNA repeat unit by exonuclease experiments⁴.

The present work can also be used to place the H2B coding sequence accurately in the partial denaturation map of cloned histone DNA. Electron microscope mapping studies revealed that the *Hind*II enzyme cleaves the double-stranded DNA region presumed to represent the H2B gene² in the right half of that DNA segment. Our present work shows that there are in fact two *Hind*II sites in close proximity to one another at positions 85 and 105, about two thirds of the way along the H2B sequence. The *Hae* III sites predicted to lie in the same area (see Fig. 1c) have now been mapped, by sequencing, near Ala 76 and 125, respectively. It should be mentioned, that the largest *Hae* III fragment has been shown, by sequence analysis, to extend into the H3 coding sequences to the right. Furthermore, the N-terminal end of the H2A coding sequence has been located using another *Hae* III fragment of known map position, so that this coding sequence also has been identified and mapped unequivocally within the cloned histone DNA of *Psammechinus* W. S. and M. B., unpublished). Our sequencing data therefore confirm our previous assignments of genes^{1,7} as well as their positions in relation to spacer DNA². Nothing can as yet be said about the extracistronic sequences since they show no homologies to known untranslated mRNA sequences.

Evolution of the H2B protein sequences

The available DNA sequences define about two thirds of the amino acid moieties of the H2B protein of *Psammechinus*. There is extensive sequence homology with both the calf thymus and the sea urchin *Paracentrotus* H2B protein, especially near the C terminus. This is in keeping with the general tenet that histones are conservative in evolution. There are some disparities, however. Thus position 78 and 100 are different in all three proteins. Two substitutions Arg $\rightleftharpoons$ Lys occur in positions 75 and 143. Val in position 142 apparently is different from the Ser in either of the two other proteins. There is a whole series of interchanges involving Val $\rightleftharpoons$ Ile. It should be noted that, with two exceptions, all amino acid substitutions are conservative and must therefore be the result of natural selection acting at the level of protein sequence within the living animal. This suggests that the change we observe reflects the evolution of the H2B sequences within the sea urchin rather than a randomisation process which might have occurred during cloning and amplification of the histone DNA in a lambda vector, and further supports the hypothesis that the cloned histone DNA has remained essentially unaltered after passage through *E. coli*.

We have asked whether the frequent substitutions Val $\rightleftharpoons$ Ile could be neutral mutations occurring by a random process. In this context it is pertinent to note that the codons in region 62, 66, 72, 79, 84, 87, 91, 107 and 112 are such that interconversion of these two amino acids could occur by substitution of a single base G $\rightleftharpoons$ A. If the appearance of A or G in the first position was determined purely by chance, there would be a 1 : 8 probability of finding only Val at a given position in all three species, a 1 : 8 chance of finding only Ile and a 6 : 8 probability of finding a mixture of the two. In the nine instances cited above the

frequency of occurrence of Val only, Ile only or Val and Ile mixed at a given site in the three species are 3 : 9, 2 : 9 and 4 : 9, respectively. These ratios are distinctly different from those expected from a stochastic process and the appearance, in many instances, of Val only, or Ile only, must be the result of natural selection at the protein level.

Considering only amino acids which have been unequivocally identified, the known sequence of *Psammechinus* differs from that of *Parechinus* in overall nine moieties, but only in seven from calf thymus H2B; while the work of the von Holt group¹⁵ has shown the calf thymus and *Parechinus* sperm H2B to differ in 11 amino acids within the sequences delimited by the present work. The known *Psammechinus* H2B sequences are more like those of calf thymus H2B than those of *Parechinus* sperm. Now, the H2B histones of sea urchins are a diverse class of proteins giving rise to variants¹⁷ which are often associated with tissue-specific gene expression. Interestingly, H2B proteins isolated from *Parechinus* embryos are more like calf thymus H2B (von Holt, personal communication) and so it is possible that the histone DNA clone we are analysing is derived from those gene units which would normally have been active during early stages of sea urchin development.

We thank Drs F. Sanger, M. Smith and N. L. Brown for instruction and hospitality during the stay of W.S. in Cambridge,

England, and Mr K. J. Rüegg, ETH Zürich, for the development of computer programs. We also thank Professor von Holt for making protein sequence information available before publication, and John Telford, Edith Bohl, Arlette Binkert and Dr Margaret Chipchase for assistance. H.O.S. was supported by the Roche Research Foundation, and W.S. received an EMBO Short Term Fellowship. The work was supported by the Schweiz. Nationalfonds, the State of Zürich, and by the US Public Health Service.

Received 7 December 1976; accepted 10 February 1977.

- ¹ Schaffner, W., Gross, K., Telford, J. & Birnstiel, M. *Cell* **8**, 471–478 (1976).
- ² Portmann, R., Schaffner, W. & Birnstiel, M. *Nature* **264**, 31–34 (1976).
- ³ Kedes, L. H. & Birnstiel, M. L. *Nature new Biol.* **230**, 165–169 (1971).
- ⁴ Gross, K., Schaffner, W., Telford, J. & Birnstiel, M. *Cell* **8**, 479–484 (1976).
- ⁵ Cohn, R. H., Lowry, J. C. & Kedes, L. H. *Cell* **9**, 147–151 (1976).
- ⁶ Weinberg, E. W., Overton, G. C., Shut, R. H. & Reeder, R. H. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4815–4819 (1975).
- ⁷ Clarkson, S. G., Smith, H. O., Schaffner, W., Gross, K. W. & Birnstiel, M. L. *Nucleic Acids Res.* **3**, 2617–2632 (1976).
- ⁸ Gross, K., Probst, E., Schaffner, W. & Birnstiel, M. *Cell* **8**, 455–469 (1976).
- ⁹ Maxam, A. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 560–564 (1977).
- ¹⁰ Sanger, F. & Coulson, A. R. *J. molec. Biol.* **94**, 441 (1975).
- ¹¹ Smith, H. O. & Birnstiel, M. L. *Nucleic Acids Res.* **3**, 2387–2398 (1976).
- ¹² Birnstiel, M., Telford, J., Weinberg, E. & Stafford, D. *Proc. natn. Acad. Sci. U.S.A.* **71**, 2900–2904 (1974).
- ¹³ Telford, J., Boseley, P., Schaffner, W. & Birnstiel, M. *Science* **195**, 391–393 (1977).
- ¹⁴ Szybalski, W., Kubinski, H., Hradecna, Z. & Summers, W. C. *Meth. Enzym.* **21**, 383–413 (1971).
- ¹⁵ Strickland, M., Strickland, W. N., Brandt, W. F. & von Holt, C. *Eur. J. Biochem.*, submitted.
- ¹⁶ Iwai, K., Ishikawa, K. & Hayashi, H. *Nature* **226**, 1056–1058 (1970).
- ¹⁷ Cohen, L., Newrock, K. & Zweidler, A. *Science* **190**, 994–998 (1975).

letters to nature

A galactic vacuum cleaner?

A REMARKABLE feature of the distributions of CO, H₂, HI, HII and dust in the Galaxy is that their most dense regions form a broad ring-like structure surrounding the galactic centre, along with a dense nuclear disk located at the centre^{1–4}. Between the ring and the nuclear disk there exists a region of very low density. The radius of the nuclear disk is approximately 600 pc, and the ring extends from an inner radius of 2–4 kpc out to a radius greater than 15 kpc for HI, but to only 8–10 kpc for the interstellar components expected to be generated by spiral shocks (namely CO, H₂ and HII). We point out here that this structure is a possible consequence of a postulated stellar bar at the centre of the galaxy.

The difference between the HI ring and that of the other components can be explained in terms of the radial variation of the spiral shock strength, and this idea forms the basis of Stecker's recent proposal for modifying the galactic population classes⁵. The shock strength is an increasing function of the component of the gas velocity perpendicular to the assumed spiral stellar potential, namely $r(\Omega - \Omega_p) \sin i$, where r is the galactocentric radius, Ω and Ω_p are the angular velocities of the gas and spiral pattern respectively, and i is the spiral pitch angle. One way in which the shock strength could decrease to zero at 8–10 kpc, and hence explain the confinement of CO, H₂ and HII to within that radius, is for corotation ($\Omega = \Omega_p$) to occur at around 10 kpc. This would require $\Omega_p \approx 25$ (km s⁻¹) kpc⁻¹ on the basis of the Schmidt model, and some support for such a value for Ω_p comes from the shape of the HI line profile in the direction of the anticentre⁶.

Shu⁷ has suggested an explanation for the absence of HII regions in the 600 pc–3 kpc region assuming that the inner Lindblad resonance occurs at about 3 kpc. Standard spiral density wave theory predicts that there should be no stellar density wave inside this radius, and therefore no shocks to

produce the HII regions and so on. But although this could explain the deficiency of shock-generated species it does not explain why all the interstellar material is deficient there.

Here we propose an alternative explanation for the distribution of interstellar matter in the central region on the basis of a hypothetical stellar bar situated at the centre of the Galaxy. Sorensen, Matsuda and Fujimoto⁸ have computed the flow of gas in barred spiral galaxies, assuming a specified potential due to the stellar bar plus a stellar disk, and neglecting the self gravity of the gas. The results of these calculations show that two pairs of shocks are formed. One pair are straight, almost radial shocks situated on the leading side of the bar (B in Fig. 1), and the other pair are trailing spiral shocks originating near the bar–gas corotation points (S in Fig. 1). The S shocks can be identified with the trailing spiral arms in barred galaxies, while the B shocks are sometimes observed as straight, bi-symmetric dark lanes within the stellar bar⁹.

The most important aspect of the B shocks as far as the present considerations are concerned is that they induce a strong inflow of gas, so that almost all the gas originally occupying the region within the corotation points is swept into a dense nucleus at the centre of the bar. The physical reason for this is that the gas plunges into the B shocks almost at normal incidence leading to a very strong shock (in contrast to the weaker shocks in ordinary spirals with oblique incidence). The gas loses both angular momentum and energy (by radiation), and consequently falls towards the centre after the shock, slowly spiralling inwards as it successively traverses the shocks. The gas flow through the S shocks is more complicated with infall produced in the inner part of the shock while outside the corotation point the gas gains angular momentum in passing through the shock density peak and spirals outwards, although much more slowly than the infall produced by the B shocks due to the higher obliquity of incidence. In this manner the stellar bar acts as a 'vacuum cleaner' sweeping gas

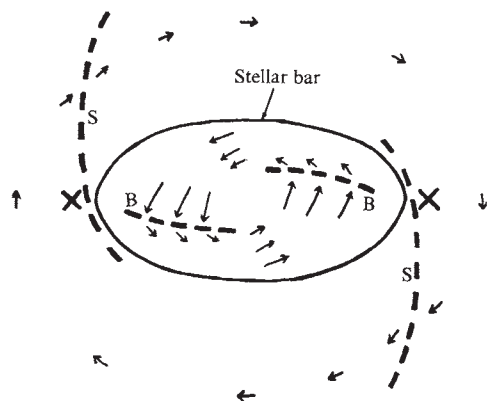


Fig. 1 Schematic diagram of the circulation of gas in a barred galaxy from the calculations of Sorensen, Matsuda and Fujimoto⁸. Arrows represent the velocity of the gas in direction and magnitude in the rest frame of the bar, the density peaks produced by the shocks are shown by the broken lines, and the crosses represent the bar-gas co-rotation points.

and dust away from the corotation region. Observations¹⁰ of HI in barred spirals support this result in that a dense nucleus is observed at the centre of the bar along with an extended ring coincident with and extending beyond the optical spiral arms.

A small central bar at the centre of ordinarily spiral galaxies has been previously proposed in a few contexts. Mishurov and Suchkov¹¹ have suggested that spiral density waves may be excited by a rotating central bar, while Contopoulos¹² has shown from the calculation of stellar orbits that a gravitationally self-consistent bar can be obtained inside the inner Lindblad resonance. From the observational viewpoint attempts¹³ have been made to explain the radial motions of HI at the galactic centre in terms of elliptic orbits which could possibly be induced by a bar. Observations of Cohen and Davies¹⁴ are not inconsistent with this explanation of the radial motions, and furthermore the geometry of the HI features which they have obtained are highly suggestive of a stellar bar in that their features IIIa and XIVa would correspond to the B shocks, while features XII and I would correspond to the S shocks. If the steep drop in the interstellar densities at 2–4 kpc represents the outer extent of the in-sweeping effect of a bar then the corotation point of the gas with respect to the bar should occur at approximately this radius. This is in reasonable agreement with the extent of features IIIa and XIVa, which should lie within the corotation points, and in fact extend out to 2 kpc.

If the bar corotates with the spiral wave pattern in the outer part of the Galaxy at say $25 \text{ (km s}^{-1}\text{) kpc}^{-1}$ then we would require a deep minimum in the rotational velocity of the gas somewhere in the region of 2 kpc from the centre in order to have a bar-gas corotation point there. This is inconsistent with the Rougoor–Oort rotation curve¹⁵; however the presence of strong radial motions in the central part of the Galaxy must make the definition and evaluation of the rotational velocity there uncertain, and the rotational velocities of some of the central features obtained by Cohen and Davies suggest at least the possibility of a deep minimum between 1 and 2 kpc. Such a rotational velocity minimum is observed in M31 (ref. 16), and it is noteworthy that a ring-like distribution plus a dense central nucleus for interstellar material in M31 is indicated by the combined HI and radio continuum observations.

On the other hand it is not obvious that the bar needs to co-rotate with the spiral pattern; the two may be dynamically independent with the bar rotating more rapidly than the spirals. A bar-gas co-rotation point could then be obtained at a radius of say 2 kpc even with the Rougoor–Oort rotation curve. For example, in the central part of NGC4314 tiny

spirals appear which seem to be independent of the grand design⁹.

In summary the presence of a small bar at the centre of our galaxy could provide a single explanation for three puzzling aspects of galactic structure, namely the strong central radial motions, HI features IIIa and XIVa, and the region between 600 pc and 2–4 kpc deficient in interstellar matter. We can estimate the mass of the bar under the assumption that it is the most massive constituent within the hypothetical bar-gas co-rotation point at approximately 2 kpc. This leads to a value less than a tenth that of the total galactic mass, which should be compared with the situation in normal barred spirals where the mass of the bar is probably a much more substantial fraction of the total mass.

This work was supported in part by the SRC. T. M. thanks Professor N. C. Wickramasinghe for hospitality.

T. MATSUDA
A. H. NELSON

Department of Applied Mathematics and Astronomy,
University College,
Cardiff, UK

Received 26 August 1976; accepted 1 March 1977.

- ¹ Rougoor, G. W. *Bull. Astr. Inst. Neth.* 17, 381 (1964).
- ² Scoville, N. Z., Solomon, P. M. & Jefferts, K. B. *Astrophys. J. Lett.* 187, L63 (1974).
- ³ Scoville, N. Z. & Solomon, P. M. *Astrophys. J. Lett.* 199, L105 (1975).
- ⁴ Burton, W. B. *et al. Astrophys. J.* 202, 30 (1975).
- ⁵ Stecker, F. W. *Nature* 260, 412 (1976).
- ⁶ Nelson, A. H. & Matsuda, T. *Mon. Not. R. astr. Soc.* (in the press).
- ⁷ Shu, F. H. *Am. Scient.* 61, 524 (1973).
- ⁸ Sorensen, S. A., Matsuda, T. & Fujimoto, M. *Astrophys. Space Sci.* 43, 491 (1976).
- ⁹ Sandage, A. *The Hubble Atlas of Galaxies*, 44–46 (Carnegie Institution of Washington, 1961).
- ¹⁰ Sancisi, R. in *La Dynamique des Galaxies Spirales, Colloque International CNRS* 241, 403 (1975).
- ¹¹ Mishurov, L. S. & Suchkov, A. A. *Soviet Phys. Usp.* 17, 85 (1974).
- ¹² Contopoulos, G. in *La Dynamique des Galaxies Spirales, Colloque International CNRS* 241, 21 (1975).
- ¹³ Simonson, S. C. & Mader, G. L. *Astr. Astrophys.* 27, 337 (1973).
- ¹⁴ Cohen, R. J. & Davies, R. D. *Mon. Not. R. astr. Soc.* 175, 1 (1976).
- ¹⁵ Rougoor, G. W. & Oort, J. H. *Proc. natn. Acad. Sci. U.S.A.* 46, 1 (1960).
- ¹⁶ Rubin, V. C. & Ford, W. K. *Astrophys. J.* 159, 379 (1970).

Mass distribution of massive meteoroids determined by occurrence of brilliant fireballs

DETERMINATION of the mass distribution function of bodies is of prime importance for an understanding of the evolutionary processes that determine the population of the Solar System. We report here a method for measuring mass distribution of meteoroids, by determining the occurrence frequency of very brilliant fireballs. In a certain mass regime the number distribution can be described by a power law

$$dn = (\text{const.})m^{-s} dm$$

where dn is the number of particles having a mass between m and $m+dm$ and s is the mass distribution index. s is a slowly varying function of mass, and values of s have been well established for meteoroid particles $10^{-12} < m \lesssim 10^1 \text{ g}$ by various techniques, and reasonable estimates are available for large bodies $m \gtrsim 10^5 \text{ g}$ (meteorites and asteroids). There is, however, a lack of information in the mass range $10^1 \lesssim m \lesssim 10^5 \text{ g}$ corresponding¹ to meteoroids which yield meteors of visual magnitude $-2 \gtrsim M_v \gtrsim -12$ ranging from bright visual objects to very brilliant fireballs (the term fireball generally refers to any meteor brighter than Venus, $M_v \approx -4$). The problem is one of sampling: while meteor (radar, visual, optical) counts over periods of hours at a single station are suitable for estimates of s for $m \lesssim 1 \text{ g}$ ($M_v \gtrsim 0$), such a procedure yields a prohibitively low count for larger objects. This difficulty would be overcome by observing over a large area of the Earth's surface for many years. We determine here the occurrence frequency of very brilliant fireballs, as a method of s measurement. A fireball of magnitude equal to that of the full Moon is an outstanding spectacle likely

to be well reported in an area with a high population density. With good communications and active research groups analysing reports and recording data, a reliable estimate of the influx of such large objects could be secured. In the British Isles, extensive records of fireball events covering more than 40 yr, are available^{2,3}. Most observers' estimates were based on the great illumination produced on the landscape or inside buildings, and the personnel analysing the reports were experienced workers in the field. A detailed examination of the reports reveals a consistent rate of about two lunar fireballs (apparent visual magnitude equal to or brighter than that of the full Moon) seen in the British Isles per year. Specifically, in the period 1900–36 the average occurrence frequency was 2.4 yr^{-1} . This frequency can be compared with that of visual meteors to obtain an estimate of s .

Assuming that meteor luminosity is proportional to pre-entry meteoroid mass, m , the cumulative flux N of meteors having magnitudes equal to or less than absolute magnitude, M is given by

$$\log_{10} N = (\text{const.}) - ((s-1)/2.51)M$$

For a single observer the average rate of sporadic meteors $M_v \leq 0$ is 0.86 h^{-1} (ref. 1), and as s is known⁴ to be close to 2.35 for $+4 \leq M_v \leq -2$, the cumulative rate for $M_v \leq -2$ is $7.34 \times 10^{-2} \text{ h}^{-1}$. Estimating the surface area viewed from the British Isles as $4 \times 10^6 \text{ km}^2$, then with an area of $1.16 \times 10^6 \text{ km}^2$ under surveillance by a single observer¹, the average rate for the geographical area is $2.52 \times 10^{-1} \text{ h}^{-1}$.

Of the lunar fireballs during the 36-yr period, 85% were observed between 1800 and 0200 UT, with 50% occurring 2000–2300 UT (reflecting an observation bias rather than any true variation). The effective observing period is close to 8 h d^{-1} ; also for this period the visual sporadic rate is close to the diurnal mean value. Although clear skies are not necessary for the detection of such brilliant objects, detection conditions depend upon the prevailing cloud type. If a cloud cover of $< 7/10$ is required for detection, however, the percentage time available in the British Isles is⁵ about 40%. Hence the average lunar fireball occurrence frequency becomes $2.05 \times 10^{-3} \text{ h}^{-1}$. As the height of maximum luminosity is lower for brighter meteors, the apparent magnitude $M_v \approx -12$ of a lunar fireball needs correction. From the beginning and end heights of lunar fireballs, and noting that the maximum luminosity for bright objects occurs about three-quarters of the way along the trail⁷, a mean height of 60 km is found. The absolute visual magnitude (referred to a standard height of 100 km) is therefore $M_v \approx -11$. This implies that the observed fireballs are nine stellar magnitudes brighter than meteors of $M_v = -2$.

If meteors which differ in absolute magnitude by ΔM have occurrence frequencies of N_1 and N_2 , then

$$s = 1 + (2.51/\Delta M) \log_{10}(N_1/N_2)$$

leading to $s = 1.58$. The reported mean annual lunar fireball rate of 2.4 showed no systematic variation over the 36-yr period, and it seems unlikely that this value would have an uncertainty exceeding 50%. With independent sightings of a large number of fireballs, systematic magnitude errors would not be expected, and it is not unreasonable to assign an uncertainty in ΔM of $\pm 1 \text{ mag}$. The estimate of the mass distribution index, s , is thus quite good: the uncertainty in N_2 leads to an error of 0.05 in s , and the uncertainty in ΔM implies an error of 0.06. We conclude that $s = 1.58 \pm 0.08$. The observed fireball rate is incompatible with large values of s suggested by some authors: a value of $s = 2.34$ appropriate to visual meteors implies only one lunar fireball sighting in the British Isles every 200 yr.

W. J. BAGGALEY

Physics Department,
University of Canterbury,
Christchurch, New Zealand

Received 29 November 1976; accepted 10 February 1977.

¹ Hughes, D. W. *Mon. Not. R. astr. Soc.* **166**, 339–343 (1974).

² *Observatory* **24–59** (1900–36).

³ *Mem. Br. astr. Ass.* **32**, part 1 (1936).

⁴ Millman, P. M. *The Moon* **8**, 228–240 (1973).

⁵ Brooks, C. E. P. *Mem. R. met. Soc.* **1** (10), 127–138 (1930).

⁶ Denning, W. F. *Mon. Not. R. astr. Soc.* **72**, 423–451 (1912).

⁷ McKinley, D. W. R. *Meteor Science and Engineering*, 130 (McGraw-Hill, New York, 1961).

Gravitational radiation from freely-falling bodies

THE problem of determining the gravitational radiation from freely-falling bodies is almost as old as general relativity itself. Investigators have deduced an energy loss, an energy gain and no radiation at all¹. Although Eddington² foresaw the difficulties a half century ago, many now believe that the Einstein quadrupole formula correctly describes the rate of energy loss³. Cooperstock initiated a new approach to the problem by considering two bodies in an initially static configuration by virtue of an intervening strut⁴. The strut was severed and the dynamical perturbation to the gravitational field was analysed. During the stress-breaking period, the radiated energy loss agreed with the quadrupole formula. During free fall, however, the model with two point masses proved inadequate and an interior metric was required. Even then, there were indications of a breakdown of the quadrupole formula and a much larger energy flux. A model of realistic sources has now been constructed and the previous indications have been realised. The free-fall energy loss rate exceeds that which would be anticipated by the quadrupole formula by a factor of $(a/\rho_0)^4$, where ρ_0 is the characteristic size of the bodies and $2a$ is their separation.

The model is chosen to consist of two perfect fluid spheres of radius ρ_0 with thin retaining skins. If the zero order density and pressure are

$${}^0\varepsilon = \text{constant}, {}^0P = 0 \quad (1)$$

then the metric within the spheres and strut can be expressed in the form⁵

$$ds^2 = e^{2\nu} dt^2 - e^{2(\gamma-\nu)} (dr^2 + dz^2) - r^2 e^{2(\mu-\nu)} d\phi^2 \quad (2)$$

where μ is of order G^2 . From the Einstein field equations, it follows that ν and γ are of the order G and G^2 , respectively, in the sources, as they are in vacuum. The field equations imply that ν satisfies the Poisson equation

$$\nabla^2 \nu = 4\pi G T_4^4 \quad (3)$$

From equations (1) and (3) and the conservation laws

$$\mathbf{T}^{ik};_k = 0 \quad (4)$$

the first order static pressure is found.

In a short time interval, counterstresses are applied to remove the supporting strut and envelopes, and free fall begins. The metric is then described as a second and third order perturbation of the background metric (equation (2)). The second order dynamic metric is found to have the required continuity and to be singularity-free only when the complementary phenomena of stress-breaking in the strut, pressure variation in the bodies and the ensuing free-fall motion are properly synthesised. Using the field equations, it is possible to express one of the elements b' of the third order perturbation in an inhomogeneous wave equation with the first and second order fields and the dynamic energy-momentum tensor $\mathbf{T}^{ik}$ as sources. This lengthy equation may be expressed symbolically in the form

$$\square b' = (\mathbf{T}) + (\nu)(b) \quad (5)$$

where $(\mathbf{T})$ represents terms which are linear in components of the energy-momentum tensor and $(\nu)(b)$ represents the nonlinear

terms which are functions of products of the first-order background metric and the second-order dynamic perturbation.

It was shown⁴ that the source terms above which neglect nonlinearities, yield precisely the energy flux of the quadrupole formula. For free fall, attention must also be focused on the remaining nonlinear contributions to b' . The analysis of the contributions from the many source terms is very lengthy and difficult and will be fully delineated in another publication⁶. As anticipated⁴, certain parts of the source for b' combine to give the dominant asymptotic contribution

$$b' = -4 \frac{G^3 m^3}{\alpha^2 \rho_0^2} \frac{(t-\rho)^2}{\rho}$$

where ρ is the radial coordinate. This yields a gravitational energy flux

$$\frac{dE}{dt} = -16 \frac{G^6 m^6}{\alpha^4 \rho_0^4} (t-\rho)^2$$

during the early stages of free fall.

Unlike electrodynamics, the radiated energy is dependent upon the structure of the sources. This is not surprising because of the important role of nonlinearities which are absent in electrodynamics. Indeed, it was shown⁴ that the linear terms alone imply a structure-independent radiation.

For a given separation and mass, the flux increases with diminishing size ρ_0 . This is understandable because of the increased background field intensity, which contributes through the nonlinearities to the radiation. It is to be emphasised that the expression for the flux loses its validity if ρ_0 is allowed to become so small as to violate the weak-field condition.

The harmonic coordinate condition, which has caused others difficulty for asymptotic calculations, has not been employed. Moreover, the problem of avoiding the intrusion of incoming radiation is solved by the development from an initially static configuration.

The usual derivation of the quadrupole formula treats the retarded integrals as for compact sources in electrodynamics. Moreover, various surface integrals containing the energy-momentum pseudotensor are discarded on application of the Gauss theorem. These procedures are justified both in electrodynamics for compact sources and in general relativity when the compact sources dominate the field sources. During free fall, however, the field sources are of the same order as the compact sources and the analysis leading to the quadrupole formula is inapplicable.

This work was supported by grants from the National Research Council of Canada and the University of Victoria.

F. I. COOPERSTOCK
P. H. LIM

Department of Physics,
University of Victoria
Victoria, B.C.,
Canada V8W 2Y2

Received 23 November 1976; accepted 31 January 1977.

- 1 Bonnor, W. B. *Br. J. appl. Phys.* **14**, 555 (1963).
- 2 Eddington, A. S. *The Mathematical Theory of Relativity*, 251 (Cambridge University, 1965).
- 3 Infeld, L. & Michalska-Trautman, R. *Ann. Phys.* **55**, 561 (1969); **55**, 576 (1969).
- 4 Cooperstock, F. I. *Phys. Rev. D* **10**, 3171 (1974); *Gen. Rel. Grav.* **6**, 91 (1975).
- 5 Synge, J. L. *Relativity: The General Theory*, Ch. VIII (North-Holland, Amsterdam, 1960).
- 6 Cooperstock, F. I. & Lim, P. H. *Phys. Rev. D* **15** (in the press).

Stable photoelectrochemical cells for the splitting of water

FOLLOWING Fujishima and Honda's suggestion of water photolysis by a photoelectrochemical cell¹, no stable cell has been reported (but compare refs 2, 3) capable of water decomposition

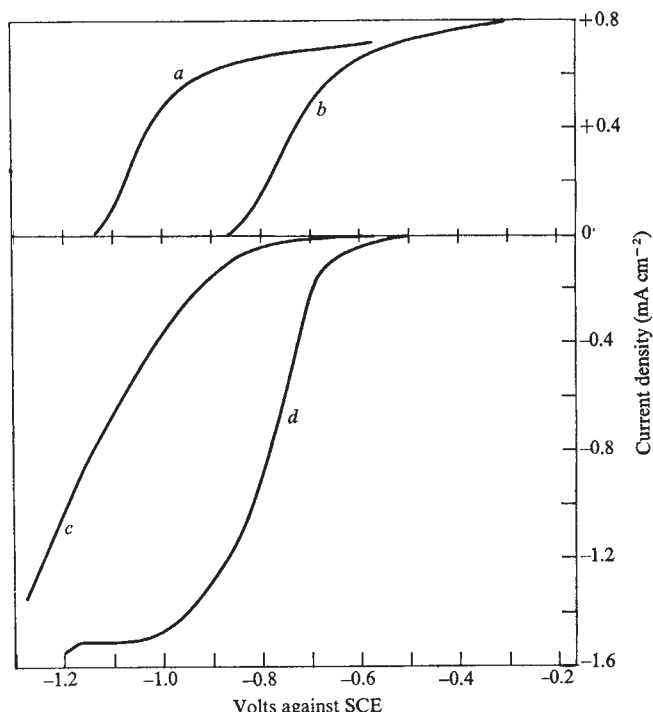
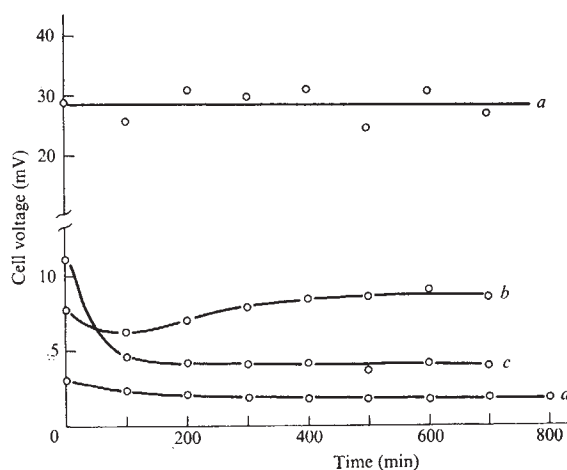


Fig. 1 Photocurrent-potential curves of semiconductor electrodes against Pt in 1 M NaOH, 25 °C. *a*, *n*-SrTiO₃; *b*, *n*-TiO₂; *c*, *p*-CdTe; *d*, *p*-GaP. Potential sweep rate: 10 mV s⁻¹. Areas: *n*-TiO₂, *p*-CdTe and *p*-GaP, 0.0491 cm², *n*-SrTiO₃, 0.0594 cm², 0.05 W cm⁻².

without the application of either an external bias or a pH gradient. The main problems have been to find stable anodes and cathodes with sufficiently negative and positive critical photopotentials, respectively, so that they may be suitably combined in a self-driving photo cell producing hydrogen and oxygen from water. We report here that the anodes *n*-TiO₂ and *n*-SrTiO₃ and the cathodes *p*-CdTe and *p*-GaP can be combined to form four separate stable self-driven cells: *n*-TiO₂-*p*-CdTe, *n*-TiO₂-*p*-GaP, *n*-SrTiO₃-*p*-CdTe and *n*-SrTiO₃-*p*-GaP capable of water photoelectrolysis.

TiO₂ (001 face) and SrTiO₃ polished single crystals were reduced in H₂ at 800 and 900 °C for 5 and 3 h, respectively, to form low-resistance *n*-type crystals. Ohmic contacts to both

Fig. 2 Cell voltage-time curves of self-driven electrochemical hydrogen producers. *a*, SrTiO₃-GaP; *b*, SrTiO₃-CdTe; *c*, TiO₂-GaP; *d*, TiO₂-CdTe. External resistance 1 kΩ. Areas: *n*-TiO₂, *p*-CdTe and *p*-GaP, 0.0491 cm², *n*-SrTiO₃, 0.0594 cm² in 1 M NaOH, light intensity 0.05 W cm⁻², 25 °C.



crystals were obtained by soldering indium on to the crystals. An ohmic contact to a *p*-CdTe (undoped; 110 face) was formed by depositing gold on a silver diffused layer. An ohmic contact to *p*-GaP (Zn doped; 100 face) was formed by soldering an In-Zn alloy on to the back of the crystal. The crystals were positioned in Teflon holders and fixed with epoxy resin.

Each pair of electrodes was positioned in adjacent compartments of an electrochemical cell with a water jacket. The light from a 900-W xenon lamp was split equally with front surface mirrors of evaporated aluminium film on glass plates and used to irradiate each electrode.

The photocurrent-potential curves of the four electrodes in 1 M NaOH, light intensity 0.05 W cm^{-2} at 25°C are shown in Fig. 1. The two *n*-type electrodes *n*-TiO₂ and *n*-SrTiO₃ can be combined with both *p*-CdTe and *p*-GaP to form self-driving photoelectrolysis cells.

The stabilities of the cells were determined by measuring the cell voltage as a function of time with an external resistance of $1 \text{ k}\Omega$ (Fig. 2). The cell voltage in each case after reaching a steady-state value after some 3 h was still stable after 12 h, although there were some fluctuations due to instability in the light source. Some electrodes were used satisfactorily for a total of 50 h in various cells.

The chemical output power of each cell (Fig. 3) was calculated from the maximum cathodic current densities based on the free energy change of $\text{H}_{2(\text{g})}$ to $\text{H}_{2\text{O}(\text{l})}$, that is, $1.23 \text{ (V)} \times i$, where i is the cathodic current density.

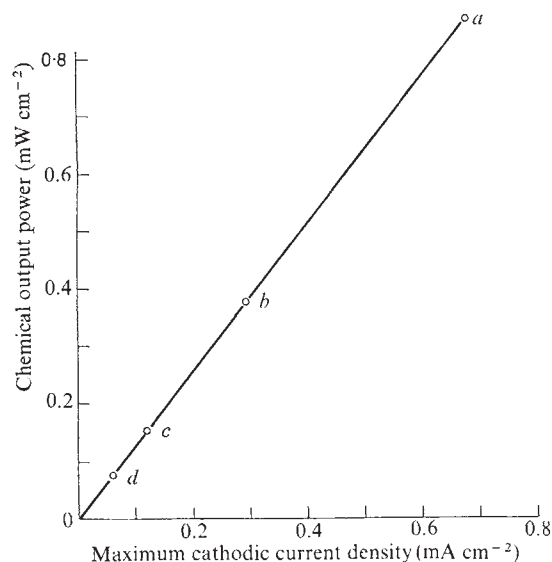


Fig. 3 Maximum cathodic current densities of self-driven electrochemical hydrogen producers and their chemical output power densities based on the free energy change of $\text{H}_{2(\text{g})}$ to $\text{H}_{2\text{O}(\text{l})}$, that is expressed as $1.23(\text{V}) \times i$, where i is the current density. *a*, SrTiO₃-GaP; *b*, SrTiO₃-CdTe; *c*, TiO₂-GaP; *d*, TiO₂-CdTe. Areas: *n*-TiO₂, *p*-CdTe and *p*-GaP, 0.0491 cm^2 , *n*-SrTiO₃, 0.0594 cm^2 . 1 M NaOH, light intensity 0.05 W cm^{-2} , 25°C .

The efficiencies of the cells in xenon light were calculated and are listed in Table 1. The efficiencies are still too low to be of practical applicability, but the difficulty is caused by the large energy gaps of the photo-anodes *n*-TiO₂ (3.0 eV) and *n*-SrTiO₃ (3.8 eV). From the energy gaps of *p*-GaP (2.25 eV) and *p*-CdTe (1.5 eV), it is possible to estimate that these electrodes can use up to 2 and 13% of the solar spectrum, respectively.

Thus, the major problem if this method is to be considered as a contender for a method of H_2 production is to find a stable anode (E_G 1.0–1.8 eV) with a critical photopotential such that it can be efficiently combined with *p*-CdTe in a self-driving

Table 1 Efficiencies of cells in xenon light*

Cell†	Efficiency in xenon light (%)
<i>n</i> -TiO ₂ - <i>p</i> -CdTe	0.044
<i>n</i> -TiO ₂ - <i>p</i> -GaP	0.098
<i>n</i> -SrTiO ₃ - <i>p</i> -CdTe	0.18
<i>n</i> -SrTiO ₃ - <i>p</i> -GaP	0.67

*Conditions: 1 M NaOH, 25°C , light intensity, 0.05 W cm^{-2} .

†Electrode areas: *n*-TiO₂, *p*-CdTe, *p*-GaP, 0.0491 cm^2 ; *n*-SrTiO₃, 0.0594 cm^2 .

hydrogen producer. The present results do not preclude the development of cells with an efficiency of $> 7\%$.

We thank M. H. Kimura for the CdTe single crystals, Dr K. Akita for the GaP single crystal and Dr D. I. Tchernev for the SrTiO₃ single crystal.

K. OHASHI

Osaka City University,
Faculty of Engineering,
Osaka,
Japan

J. McCANN

J. O'M. BOCKRIS

School of Physical Sciences,
Flinders University,
Adelaide, South Australia

Received 28 October 1976; accepted 22 February 1977.

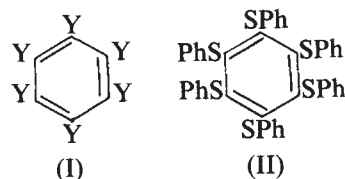
¹ Fujishima, A. & Honda, K. *Nature* **238**, 37–38 (1972).

² Mavroides, J. G., Tchernev, D. I., Kafalas, J. A. & Kolesar, D. F. *Mater. Res. Bull.* **10**, 1023–1030 (1975).

³ Watanabe, T., Fujishima, A. & Honda, K. *Bull. chem. Soc. Jap.* **49**, 355–358 (1976).

Crystal and molecular structure of a 'hexa-host' inclusion compound

We recently suggested¹, and found experimental support for^{1,2}, a totally new concept for the design of inclusion compounds. Using this design concept we prepared a series of host compounds based on a hexa-substituted benzene ring (I), and we now report the results of the first X-ray structural study on one of these compounds.



The carbon tetrachloride adduct of hexa-phenylthiobenzene (II) is trigonal with a unit cell ($a=14.263$; $c=20.717 \text{ \AA}$) containing three molecules of $\text{C}_{42}\text{H}_{30}\text{S}_6$. A host-guest (carbon tetrachloride) ratio of 1:2 was found by microanalysis for chlorine. The space group is either $R\bar{3}$ or $R\bar{3}$ (ref. 3), the centrosymmetric space group ($R\bar{3}$) was chosen and this choice has been justified by the success of the subsequent analysis. The structure was solved by direct methods using 2071 independent reflections measured with Mo-K α radiation on a Hilger-Watts automatic diffractometer (using a Lindemann capillary for crystal containment), and refined to a final R value of 0.055. All the hydrogen atoms have been located and allowed for, as have the guest molecules. Full details of the structural analysis will be published elsewhere.

Figure 1 shows a general view of the host molecule. It is centred on a point of $\bar{3}$ symmetry; thus the crystallographically equivalent 'legs' of the molecule point alternately above and

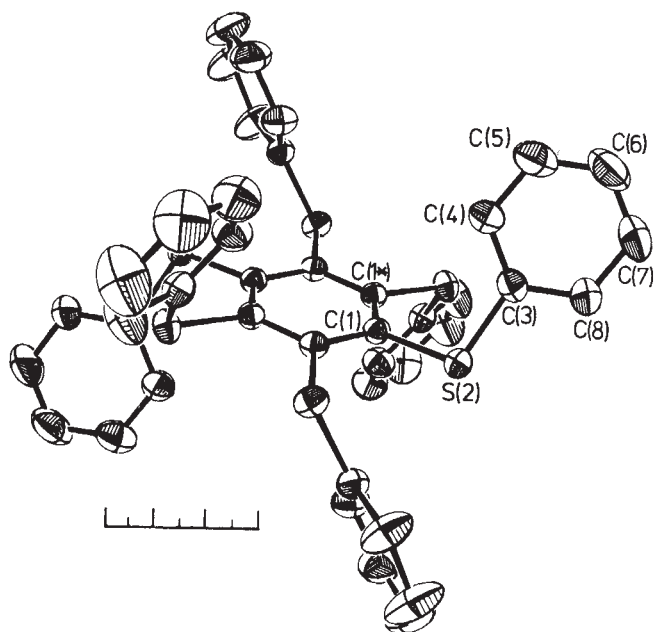
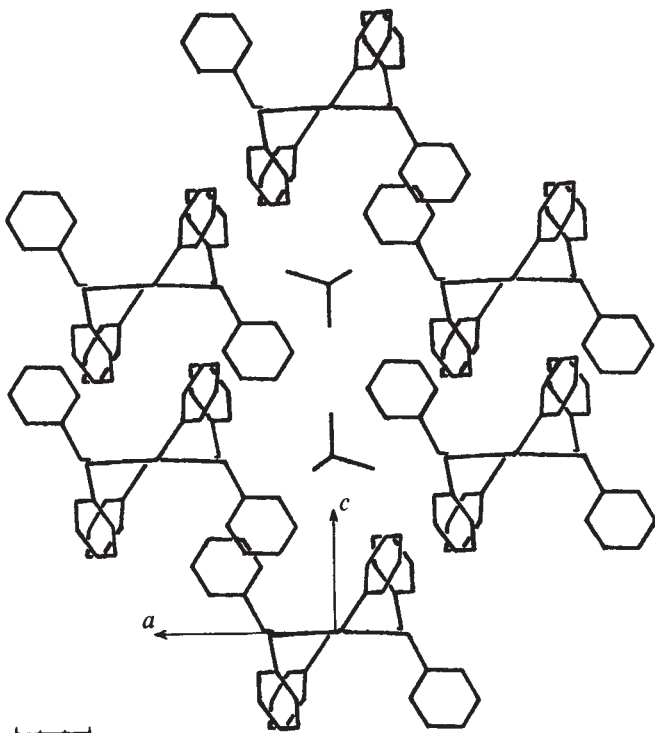


Fig. 1 An ORTEP drawing showing a general view of the molecular structure of host molecule (II) in the crystal of the CCl_4 clathrate. (The hydrogen atoms are not shown). Bar represents 3 Å.

below the plane of the central benzene ring. The relative orientation of the central and side-chain aromatic rings may be appreciated by a consideration of the torsion angles $\text{C}(1^*)\text{--C}(1)\text{--S}(2)\text{--C}(3)$ and $\text{C}(1)\text{--S}(2)\text{--C}(3)\text{--C}(4)$, which are 56° and 28° respectively.

Figure 2 illustrates the host-guest packing arrangement in the crystal; the two host molecules which complete the cage (which lie above and below the cavity as reviewed in the direc-

Fig. 2 An illustration of the host-guest packing in the crystal of the CCl_4 clathrate of (II), as viewed on to the ac plane. Two host molecules which lie above and below the cavity as viewed in this direction have been excluded to show the guest molecules more clearly. Bar represents 3 Å.



tion shown) have been omitted for clarity. The two CCl_4 guest molecules in the clathrate cage are orientated such that a C-Cl bond of each is co-linear with the c axis. Although relatively small gaps have been detected in the cage wall these are insufficiently large to allow the guest molecules to escape unhindered. Solvent loss does in fact occur slowly when the crystals are left in air at room temperature, with apparent complete disruption of the crystal lattice.

The crystal packing forces seem to be of the normal van der Waals' type, no evidence having been found to suggest marked charge transfer interactions between host and guest molecules.

A host-guest ratio of 1:2 has also been found for the guests CCl_3CH_3 and CCl_3SCl , though guest species CCl_3Br and CCl_3NO_2 form complexes with a 1:1 ratio. Recrystallisation of (II) from the solvent $\text{CCl}_3\text{CCl}_2\text{H}$ gives unsolvated triclinic crystals.

Further studies on other 'hexa-host' molecules indicate that trigonal symmetry is not always encountered in the inclusion compounds formed. A study of the complex of hexakis (benzylthiomethyl) benzene (formula (I), $\text{Y}=\text{CH}_2\text{SCH}_2\text{Ph}$) with dioxane has shown the crystals to be monoclinic, space group $\text{P2}_1/\text{c}$, with two host and two guest molecules in the unit cell.

We thank the SRC for financial support.

DAVID D. MACNICOL
ANDREW D. U. HARDY
DEREK R. WILSON

Chemistry Department,
The University,
Glasgow, UK

Received 31 January; accepted 21 February 1977.

¹ MacNicol, D. D. & Wilson, D. R. *J. chem. Soc. chem. Commun.* 494-495 (1976).

² MacNicol, D. D. & Wilson, D. R. *Chem. Ind.* 84-85 (1977).

³ *International Tables for X-ray Crystallography* (eds Macgillivray, C. H. & Rieck, G. D.) 253 (Kynoch, Birmingham, 1965).

Effect of magnetic field on reduction of iron oxides: magnetite and wüstite

A STRONG magnetic field (500-1,400 Oe) was reported by Skorski¹ to cause an increase in the reduction rate of haematite (Fe_2O_3) to metallic iron when H_2 was used as the reducing agent. This effect was attributed to the magnetic properties of the H_2 , because neither CH_4 nor CO produced a similar increase when under the influence of a strong magnetic field. Although Svare² disputed this explanation, Peters³ offered an alternative one, based on thermodynamics, which indicated that an increase in reaction rate is expected under the influence of a strong magnetic field when the reactants were relatively non-magnetic and the products were strongly magnetic—as with reduction of haematite to iron. We report here our results of studying magnetite and wüstite reduction to iron, which show an increased reduction rate for magnetite, under the influence of a strong magnetic field, but an inexplicably decreased rate for the wüstite reduction.

Confirmation of the effect noted by Skorski¹ was attempted by Rowe *et al.*⁴. To avoid the complication arising from the two-step process of haematite to iron reduction with H_2 below 550°C ($\text{Fe}_2\text{O}_3 \rightarrow \text{Fe}_3\text{O}_4 \rightarrow \text{Fe}$), Rowe *et al.* examined the simpler nickel oxide-nickel system. The $\text{NiO} \rightarrow \text{Ni}$ reduction meets Peters' criterion for expected reduction rate effects from applied magnetic fields almost as well as the $\text{Fe}_2\text{O}_3 \rightarrow \text{Fe}_3\text{O}_4 \rightarrow \text{Fe}$ system does. That is, NiO is relatively non-magnetic (paramagnetic) whereas Ni is ferromagnetic (saturation magnetisation ≈ 54). Fe_2O_3 is relatively non-magnetic (antiferromagnetic), while metallic Fe is also ferromagnetic (saturation magnetisation ≈ 218). (Fe_3O_4 is also relatively magnetic, being ferromagnetic with saturation magnetisation ≈ 92 .) Rowe *et al.* found that application of a strong magnetic

field ($\sim 4,200$ Oe) produced no detectable difference in the reduction rate of NiO to metallic Ni and therefore failed to confirm the general effect expected in the reduction of a non-magnetic species to a magnetic one.

Having observed no difference in reduction rate of NiO to Ni on the application of a strong magnetic field, we now check more directly Skorski's observation on Fe_2O_3 reduction. At temperatures less than 550°C , Fe_3O_4 is formed as an intermediate; Skorski did not attempt to account for this effect. To eliminate this complication, we decided to investigate here the reduction of magnetite and wüstite (FeO) to metallic iron. According to Peters' thermodynamic argument, the rate of reduction of FeO to Fe should be enhanced to a greater degree than expected for Fe_3O_4 to Fe, since magnetite is more strongly magnetic than wüstite. That is, the change in saturation magnetisation is greater in reducing FeO to Fe than in reducing Fe_3O_4 to Fe. Also, both of these reaction rates should be enhanced to a greater extent than in the $\text{NiO} \rightarrow \text{Ni}$ case, if Peters is correct.

Our apparatus⁴, which allows continuous observation throughout the reduction (an advantage over Skorski's 'observation after the fact' method), consists of an electrobalance (sensitive to 10^{-6} g) connected to a strip chart recorder to trace the weight of reactant (Fe_3O_4 or FeO) plus product (Fe). When the strong magnetic field is applied, the apparent weight (saturation magnetisation) of the Fe_3O_4 and Fe or the FeO and Fe are recorded. The temperature was measured by means of a thermocouple inserted nearby the quartz sample pan.

Before each run, the electrobalance system (including sample) was flushed at room temperature with 20% H_2 in N_2 carrier at a rate of 65 ml min^{-1} for at least 1 h to assure a constant reaction atmosphere for each run. The gas flow was continued until the reaction was essentially 100% complete and the experiment was ended. We used ~ 1 –3-mg samples of powdered Fe_3O_4 and FeO . The Fe_3O_4 samples were powdered to 200 mesh; finely powdered FeO samples were used but the mesh was not known. All experiments were begun at room temperature ($\sim 24^\circ\text{C}$) and reached $\sim 385^\circ\text{C}$ (for magnetite) after about

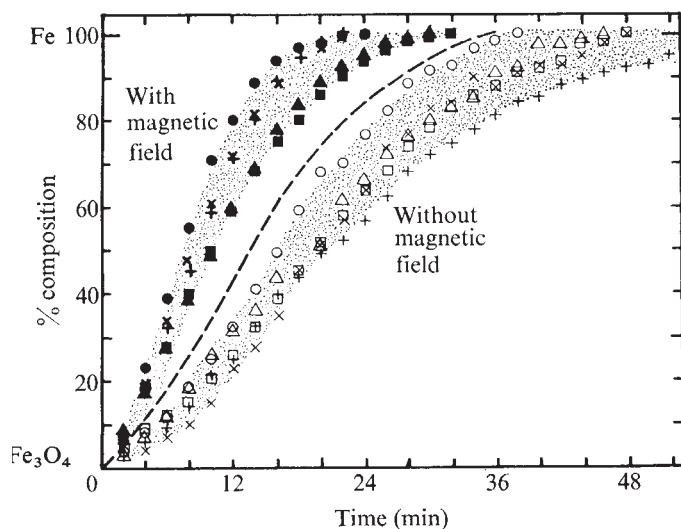


Fig. 1 Reduction of Fe_3O_4 to metallic iron with a 65-ml-min^{-1} flow of 20% H_2 in N_2 carrier at $\sim 385^\circ\text{C}$. Points were hand-read from a strip chart recording for construction of Fig. 1 so that data taken under the influence of a strong magnetic field can be directly compared with those without the strong magnetic field. The mass data were recorded continuously. Data on the left side of the dashed line in Fig. 1 were recorded after the insertion of an $\sim 4,200$ -Oe permanent magnet. Initial sample weights (Fe_3O_4): $\bullet$, 0.98 mg; $\dagger$, 0.98 mg; $\blacktriangle$, 1.29 mg; $\blacksquare$, 2.27 mg; $\times$, 1.42 mg. Data on the right side of the dashed line in Fig. 1 were recorded in the Earth's magnetic field (~ 0.5 Oe). Initial sample weights (Fe_3O_4): $\circ$, 1.24 mg; $+$, 1.53 mg; $\triangle$, 2.22 mg; $\square$, 1.54 mg; $\times$, 1.18 mg.

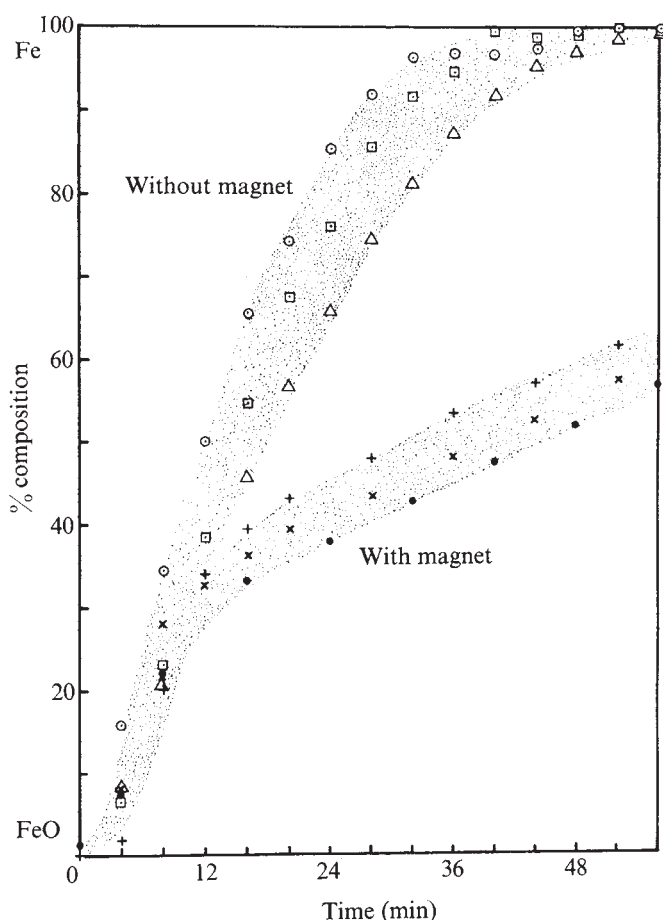


Fig. 2 Reduction of wüstite to metallic iron with a 65-ml min^{-1} flow of 20% H_2 in N_2 carrier at $\sim 490^\circ\text{C}$. Points were hand-read from a strip chart recording (see Fig. 1 legend). The mass data were recorded continuously. Data on the left side of Fig. 2 were recorded in the Earth's magnetic field (~ 0.5 Oe) those on the right side were recorded after the insertion of an $\sim 4,200$ -Oe permanent magnet. Initial sample weights (FeO): $\circ$, 1.31 mg; $\square$, 1.76 mg; $\triangle$, 2.51 mg; $\bullet$, 2.46 mg; $+$, 1.05 mg; $\times$, 2.25 mg.

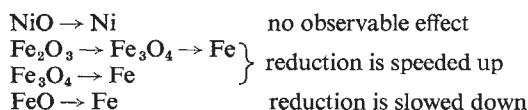
8 min, $\sim 490^\circ\text{C}$ (for wüstite) after about 10 min. Reaction did not occur until near the reaction temperature.

For magnetite, five runs were performed in the Earth's field, and five after the insertion of an $\sim 4,200$ -Oe permanent magnet (Fig. 1). The reductions were all carried to essential completion (as confirmed by stoichiometry), and the reduction rates compared. Skorski's¹ reductions were not generally carried to completion. Figure 1 shows that the reduction of magnetite to iron occurred at a significantly faster rate under the influence of the strong magnetic field, thus confirming Skorski's observation by an independent technique. This is not, however, direct confirmation because we found the reaction rate was increased on reducing Fe_3O_4 to Fe, whereas Skorski started with Fe_2O_3 and ended with an Fe_2O_3 , Fe_3O_4 and Fe mixture of unknown composition.

For the wüstite reduction, three runs were conducted with a strong magnet, and three without (Fig. 2). The reductions were all carried to (and recorded to) essential completion, as for magnetite, and the rates compared. Although the data for the wüstite reduction in the presence of the strong magnetic field are not shown to completion in Fig. 2, the difference in rates brought about by the magnetic field is apparent.

Surprisingly, we found that the application of a strong magnetic field during the wüstite reduction with H_2 resulted in a significantly slower reaction rate than when the reduction was conducted in the Earth's field. This supports our earlier suspicion that Peters's³ thermodynamic argument was not the determining factor in the reductions studied to date (refs 1, 4

and present work). In all cases, the general requirement is met that the product be more highly magnetic than the reactant, but a strong magnetic field affects the reduction rates as follows:



We are not able to explain these data at present. This work was supported by NASA.

M. W. ROWE
S. M. LAKE
R. FANICK

Department of Chemistry,
Texas A & M University,
College Station, Texas 77843

Received 11 November 1976; accepted 21 January 1977.

¹ Skorski, R. *Nature phys. Sci.* **240**, 15 (1972).

² Svare, I. *Nature phys. Sci.* **244**, 78 (1973).

³ Peters, C. T. *Nature phys. Sci.* **244**, 79 (1973).

⁴ Rowe, M. W., Fanick, R., Jewett, D. & Rowe, J. D. *Nature* **263**, 756 (1976).

Computer simulation and statistical geometric model for contacts in binary random two-dimensional disk packings

EXPERIMENTAL investigation of the characteristics of multi-component packings of particles would involve a large number of manipulations for even very few components in the mixture, and each additional component would increase this number enormously. A method of calculating the properties of such mixtures would be of interest if only as a framework within which to design experiments. One difficulty is that for this very reason a theoretical approach cannot be subjected to a thorough experimental verification. We therefore think it useful to take the problem further by comparing two previously published theoretical approaches, by a statistical geometric model¹ and a computer simulation², for calculating the contacts between disks in two-dimensional multicomponent random mixtures.

The average total coordination number in a packing is defined as the total number of contacts in the packing divided by the total number of disks in the packing (C). In multi-component packings this may be further subdivided: first, into the individual average coordination number for each disk size (c_i), that is, the total number of contacts on disks of a given size divided by the number of disks of that size; second, into the average coordination number for each size by each of the other disk sizes in the packing (p_{ij}).

In a binary packing, these various coordination numbers are related by the following expressions:

$$C = (n_1 c_1 + n_2 c_2) / (n_1 + n_2) \quad (1)$$

$$c_1 = p_{11} + p_{12} \quad (2a)$$

$$c_2 = p_{21} + p_{22} \quad (2b)$$

Note that $p_{12} \neq p_{21}$ but $n_1 p_{12} = n_2 p_{21}$

It was shown previously¹ that the average coordination number on each disk size (c_i) could be calculated from a disk size distribution (relative numbers of disks n_i and their diameters d_i) using a simple statistical geometric model of an idealised gapless packing of which the space was divided up into triangular subunits. The matrix of (p_{ij}) values can also be calculated from the model through the relative numbers of triangular subunits (T_{ijk}) calculated by expressions (3) and (4) in ref. 1. Each of these subunits includes three contacts and the relative numbers of the different types of contact between disks can therefore be calculated. For a binary mixture there are three types of contact and expressing these as fractions of the

total number of contacts we have

$$t_{11} = (T_{111} \times 3 + T_{112}) / 3 \quad (3a)$$

$$t_{22} = (T_{222} \times 3 + T_{122}) / 3 \quad (3b)$$

$$t_{12} = t_{21} = (T_{112} \times 2 + T_{122} \times 2) / 3 \quad (3c)$$

$$t_{11} + t_{22} + t_{21} = 1 \quad (4)$$

By definition and for a binary mixture

$$t_{11} = \text{number of type 11 contacts} / \text{total number of contacts}$$

$$t_{11} = (n_1 p_{11}) / (n_1 c_1 + n_2 c_2) \quad (5a)$$

$$t_{22} = (n_2 p_{22}) / (n_1 c_1 + n_2 c_2) \quad (5b)$$

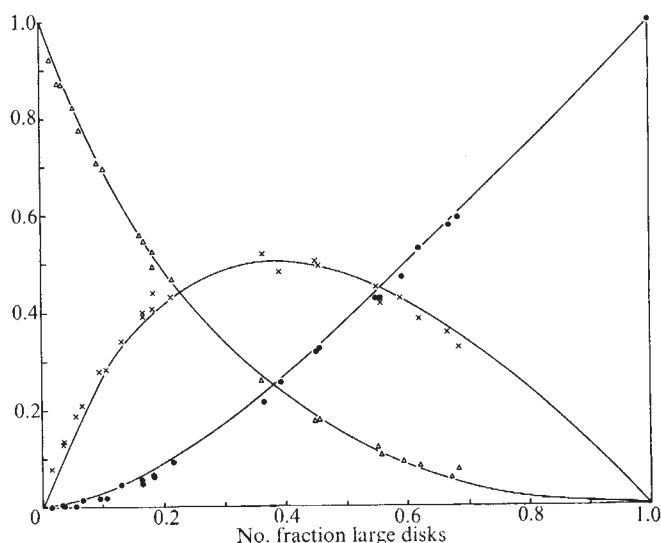
$$t_{21} = t_{12} = (n_1 p_{12} + n_2 p_{21}) / (n_1 c_1 + n_2 c_2) \quad (5c)$$

By elimination between expressions (2), (3) and (5) we may calculate values of p_{ij} .

As reported in ref. 2, the process simulated is that of disks being put into a two-dimensional box in random order from a random point and allowed to fall and roll until they attain a dynamically stable position. No adhesion or rebound is considered and once in position a disk is assumed not to move. The various coordination numbers are obtained by counting after rejection of the disks at the edge.

It is well known³ that the porosity and coordination number in packings depend not only on geometrical factors (size distribution) but also on what may be called mechanical factors such as the conditions in which the packing is formed: vibrated, settled in water dropped from a height, or the properties of the grains other than size; their density, surface roughness, existence of attractive or repulsive forces. For example, a packing of monosize glass spheres can have a porosity of 0.40 or 0.36 and an average coordination number of 8 or 9 depending on whether the packing is vibrated or not. Grains with a higher density or with attractive forces between them tend to pack to lower porosities than grains with surface roughness or repulsive forces between them. As an example of these effects it is possible to simulate a looser packing with a lower total coordination number by including an adhesional probability in the computer simulation. When a disk is put into the box and touches another disk a random number is compared with the assumed adhesional probability: if the random number is smaller than the adhesional probability the disk is fixed in that position, if it is greater the disk continues to roll until the next contact when the procedure is repeated or the disk reaches a stable position. All the results reported here are

Fig. 1 Comparison of coordination points from the model with computer simulation. Diameter ratio 2.5. Values of t_{ij} are shown as curves, and computer simulation results are shown as points: Δ , t_{11} ; $\times$, t_{12} ; $\bullet$, t_{22} .



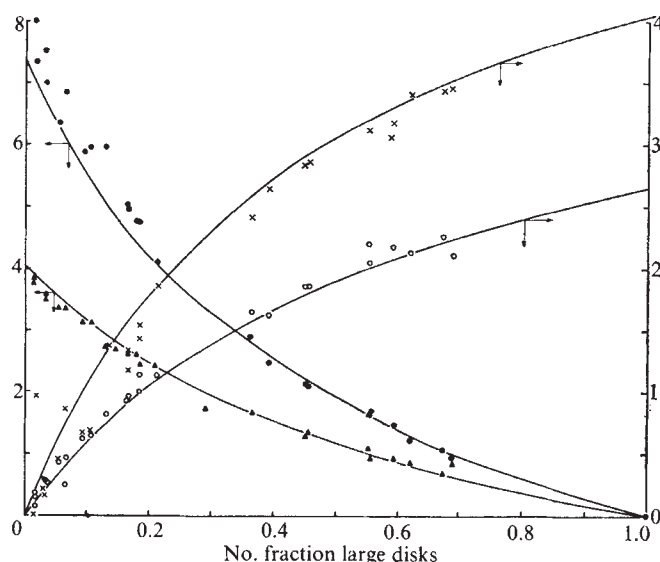


Fig. 2 Comparison of coordination points from the model with the computer simulation. Diameter ratio 2.5. Values of i_{ij} from the model multiplied by the compaction factor are shown as curves, and computer simulation results are shown as points:

▲, P_{11} ; ○, P_{12} ; ●, P_{21} ; ×, P_{22} .

for an adhesional probability of 0%. Here the model is concerned only with geometrical factors and relates to an idealised compact gapless packing, with an average total coordination number of 6. The computer simulation includes mechanical factors to a certain extent and gives values for a more realistic packing with a constant average total coordination number of 4.02 ± 0.04 for all mixtures considered here. This geometrical invariant may be used to define a 'compaction factor' which represents the degree to which mechanical factors allow real packings (here computer simulated) to approach the geometry of the idealised packing. In this case the factor is $4.02/6.00 = 0.67$. Multiplying by this factor allows the different coordination numbers obtained from the model to be compared with the computer simulation. The figures show this comparison. Figure 1 (analogous to Fig. 3 in ref. 1) shows values of i_{ij} from the model as smooth curves and results from the computer simulation as points; the agreement is excellent. Figure 2 (analogous to Fig. 6 in ref. 2) gives values of p_{ij} from the model multiplied by the compaction factor as smooth curves and the results from the computer simulation as points. The agreement is excellent which indicates the concept of the compaction factor.

The comparison between the coordination numbers for two-dimensional binary random disk packings as calculated by computer simulation and a theoretical model shows a good agreement between the two methods. Notably the invariability of the average total coordination number with size ratios and mixture proportions is confirmed. This allows the definition of a 'compaction factor' which allows the results of the theoretical model for an idealised gapless packing to be used to calculate coordination numbers for real packings.

JOHN DODDS

Laboratoire des Sciences du Génie Chimique,
CNRS-ENSIC,
1, rue Grandville,
54042 Nancy Codex, France

HIROSHI KUNO

Faculty of Engineering,
Kaio University,
Hiyoshi 832 Kohoku-ku,
Yokohama, Japan

Received 3 December 1976; accepted 11 February 1977.

¹ Dodds, J. A. *Nature* 256, 187–189 (1975).

² Kuno, H. *J. Jap. Soc. Pow. Pow. Met.* 19, 85–89 (1972).

³ Gray, W. A. *The Packing of Solid Particles* (Chapman and Hall, London, 1968).

Gravity in Zambia

A RECONNAISSANCE gravity survey of the Republic of Zambia was carried out from 1971 to 1975 by the Geological Survey of Zambia in collaboration with the University of Zambia and The University of Michigan¹. We present here a Bouguer anomaly map and preliminary analysis of the gravity data, in the context of the principal tectonic elements and structural characteristics of eastern and southern Africa.

Zambia is situated on the Central African Plateau which slopes gently from an elevation of 1,000 m in the south and west of Zambia to a maximum elevation of >2,000 m in the north-east. The characteristic gently sloping terrain is broken in the extreme north by the Tanganyika and Mweru rifts and in the east and south, respectively, by the Luangwa and mid-Zambezi rifts, which contain Karroo (Permo-Triassic) sediments and volcanic rocks. The plateau comprises principally Pan-African (450–750 Myr) and older igneous and metamorphic basement rocks. In the west, the basement is in places broken by fault block basins, but the distribution of these is obscured by a blanket of Quaternary Kalahari sands.

Over 2,700 gravity stations were established along the primary and secondary road network of Zambia, at approximately 10–15 km intervals, with a characteristic density of one station per 40 km². All stations were referenced to the previously established East African primary net². Free-air and simple Bouguer anomalies were calculated using the 1930 International Gravity Formula and a density of 2.67 g cm⁻³. Elevations for about one-third of the stations were taken from topographic map contours; at the remaining stations, elevations were determined with aneroid barometers. A mean uncertainty of ± 2 mgal, arising principally from uncertainties in elevation, is typical.

Our Bouguer anomaly map of Zambia (Fig. 1) shows Bouguer anomalies ranging between –200 and –75 mgal, with a mean near –140 mgal. The regions in the north and northwest marked by the –160-mgal contour lie along the axis of the so-called Great Negative Bouguer Gravity Anomaly³ of Africa; the long and continuous –120-mgal contour in the south marks its approximate southern boundary. The overall grain of the map is dominantly northeast–southwest, a tectonic trend which is prominently displayed by the Upemba graben in southern Zaire, the Mweru and Luangwa rifts, the middle Zambezi valley, and which continues to the south-west into northern Botswana⁴.

Some of the individual features of the map can be clearly identified with structural elements, the best example being the elongated gravity trough in eastern Zambia which coincides closely with the Luangwa rift. The southern terminus of the Tanganyika rift, and the northern margin of the Zambezi rift along Lake Kariba also show gravity depressions. The arcuate trend of the –140-mgal contour in the northern part of central and western Zambia follows closely the curvature of the Lufilian arc, a Pan-African age structural element that hosts the rich copper mineralisation of Zambia and southern Zaire. The moderate gravity depression in central western Zambia is associated with a zone of persistent seismic activity⁵; the large nearly circular depression in northern Zambia lies within a region of Precambrian platform sediments, and could represent a small sedimentary basin.

Three regions show prominent gravity highs: the eastern border with Malawi, the region south of Lake Mweru, and the region north of Lake Kariba. In each of these areas the anomalies reach –80 mgal with gravity relief of at least 40 mgal. These highs are not associated with any obvious structural elements or topographic settings; they are in areas with elevations of 900–1,800 m.

Residual anomalies were obtained by subtracting a smooth regional anomaly field from the observed Bouguer anomaly field. The regional pattern was estimated by a number of

techniques, including free-hand smoothing, Fourier and polynomial surface-fitting methods, and a mean anomaly against elevation parameterisation. Each representation of the regional field yielded different residuals, but some anomalous regions were common to all of the residual fields. Those regions, each with residual anomalies > 20 mgal (inset map of Fig. 1) display a north-east-south-west grain, as does the anomaly map. The most prominent feature is the chain of positive residuals extending from south-western Zambia north-eastward along the southern margin of the country. This remarkable feature merely traverses Zambia; it can be traced south-westward through Botswana to the Namibia border⁴, and continues north-eastward into the Malawi highlands⁶, approaching 2,000 km in length. This prominent positive residual buttresses the southern margin of the Great Negative Anomaly throughout central Africa.

The negative residual over the Luangwa rift arises from the low-density Karroo sedimentary fill. A similar residual probably exists in the mid-Zambezi valley beneath Lake Kariba, but it has not been confirmed due to a lack of data from the valley and beyond in Rhodesia. The negative residual which extends from the north across the Zaire pedicle into the Zambian Copperbelt possibly reflects relatively thick sedimentary accumulations in the north, but is associated with the Copperbelt granites at its south-western terminus.

A scatter plot of Bouguer anomalies against elevation is shown in Fig. 2a. Stations at < 800 -m elevation lie exclusively within the Luangwa, Zambezi and Tanganyika troughs, and

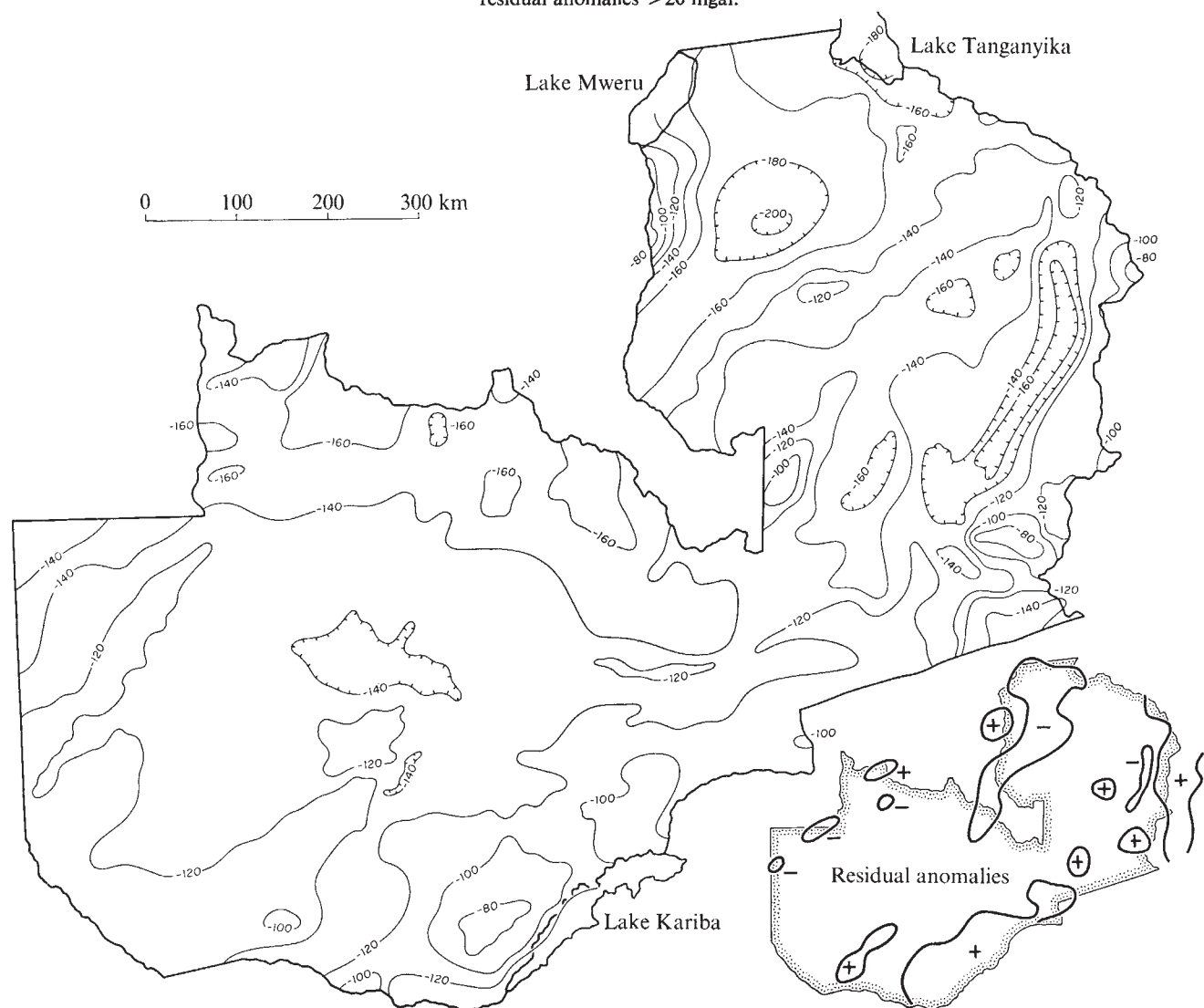
thus afford an opportunity to compare gravity variations in rift and non-rift settings. Regression lines were fitted to mean Bouguer anomalies and mean elevations in each 100-m elevation interval; these lines are shown in Fig. 2 and their parameters in Table 1, with similar, earlier analyses from east Africa^{7,8}.

The Zambia and Kenya regression lines have similar slopes in their respective rift and non-rift settings, but the Zambian intercepts in both settings are substantially more negative. The Zambian line for non-rift stations is almost the same as that determined by Woollard for $3^\circ \times 3^\circ$ mean anomalies in east Africa.

The different slopes in rift and non-rift areas implies different densities in the two settings, and suggests contrasting crustal and upper mantle structures. The significance of the different intercepts is unclear, but the differences may stem from the fact that in Woollard's⁸ and our analyses, the lines were fitted to mean anomalies, while Khan and Mansfield⁷ presented a subset of their individual station data, which may be biased away from the regional mean.

Free-air anomalies are also of interest because they can give to a first order an estimate of how closely a region approaches isostatic equilibrium. When isostasy prevails, free-air anomalies are usually less than a few tens of mgal, distributed randomly about zero, and show very little dependence on elevation. Woollard⁸ gives an empirical relationship between free-air anomalies and elevation in isostatic regions as $FA = 0.0075h - 6$, where FA is the free-air anomaly in mgal and h is the elevation in m.

Fig. 1 Bouguer gravity anomaly map of Zambia. Contour interval 20 mgal. Reduction density 2.67 g cm^{-3} . Inset map shows residual anomalies > 20 mgal.



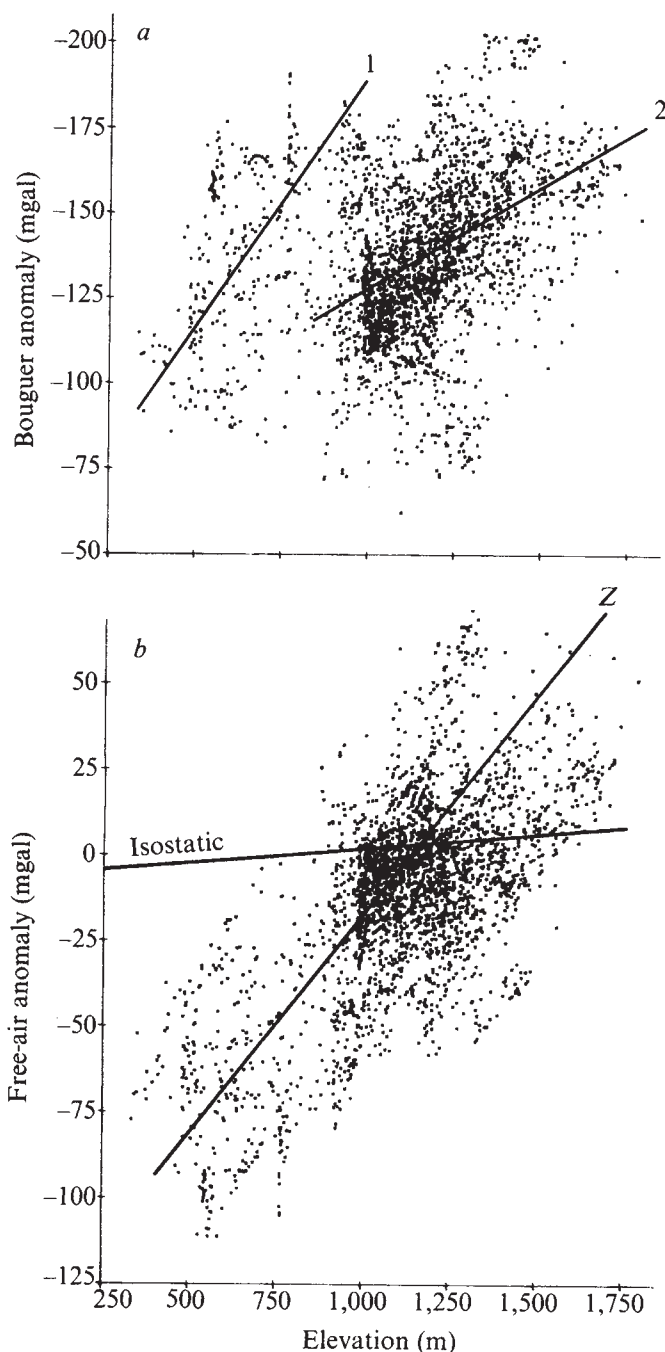


Fig. 2 *a*, Scatter plot of Bouguer anomalies against elevation, with regression lines for rift (1) and non-rift (2) stations. *b*, Scatter plot of free air anomalies against elevation. Line labelled Z represents Zambian data; line labelled 'Isostatic' represents empirical relationship of Woollard⁸. Both lines are fitted to mean anomalies and elevations within 100-m intervals.

Figure 2*b* is a scatter plot of free-air anomalies against elevation for Zambia, and shows a clear departure from the isostatic relationship and an obvious dependence of anomalies on elevation. Such a dependence of free-air anomalies on elevation is not uncommon in regions of considerable relief, but in Zambia, apart from the escarpments adjacent to the rifts, the terrain is very gentle.

It is, therefore, possible that the Central African Plateau may not be in isostatic equilibrium. Woollard⁸ notes that a plateau undergoing uplift can exhibit a regional departure from isostasy. Considerable evidence is accumulating that suggests that this part of central Africa is dynamic and responding to tectonic stress. Widely distributed earthquake epicentres⁵, tensional focal mechanisms^{9,10}, anomalous heat flow¹¹ and disrupted drainage

Table 1 Regressions of Bouguer anomalies (mgal) on elevation (*h*, m)

	Zambia	Kenya ⁷	East Africa ⁸ (3° × 3° mean anomalies)
Rift	-0.15 <i>h</i> -41	-0.137 <i>h</i> -4.31	
Non-rift	-0.06 <i>h</i> -67	-0.082 <i>h</i> -5.85	-0.063 <i>h</i> -57

as shown on satellite photography together make an interesting if not compelling case for contemporary tectonism (incipient rifting) in the Central African Plateau. In such a context, it seems possible that isostasy is yet to be fully established in this current phase of tectonism. If tensional block faulting continues, however, and becomes more widespread in the Central African Plateau, a restoration of isostatic equilibrium will probably follow.

We thank A. R. Drysdall and O. Mazac of the Geological Survey of Zambia for making data available. Financial support was provided by the Universities of Zambia and Michigan, and the US NSF.

IAIN M. COWAN

School of Natural Sciences,
University of Zambia,
Box 2379,
Lusaka, Zambia

HENRY N. POLLACK

Department of Geology and Mineralogy,
The University of Michigan,
Ann Arbor, Michigan 48109

Received 13 September 1976; accepted 27 January 1977.

- ¹ Geol. Surv. Dep. Zambia, Tech. Rep. No. 76 (1974).
- ² Masson Smith, D. J. & Andrew, E. M. *Geophys. J. R. astr. Soc.* 7, 65-86 (1962).
- ³ Girdler, R. W. *EOS Trans. Am. geophys. Union* 56, 516-519 (1975).
- ⁴ Reeves, C. V. & Hutchins, D. G., *Nature* 254, 408-410 (1975).
- ⁵ International Seismological Centre Regional Catalogue of Earthquakes, Edinburgh (1964-1973).
- ⁶ Andrew, E. M. *Inst. Geol. Sci. Rep.* 74/15 (1974).
- ⁷ Khan, M. A. & Mansfield, J. *Nature phys. Sci.* 229, 72-75 (1971).
- ⁸ Woollard, G. P. *Am. geophys. Union Monogr.* 13, 320-341 (1969).
- ⁹ Fairhead, J. D. & Girdler, R. W. *Geophys. J. R. astr. Soc.* 24, 271 (1971).
- ¹⁰ Scholz, C. H., Koczyński, T. A. & Hutchins, D. G. *Geophys. J. R. astr. Soc.* 44, 135-144 (1976).
- ¹¹ Chapman, D. S. & Pollack, H. N. *Nature* 256, 28-30 (1975).

Electrical conductivity and tectonics of Scotland

A REGION of anomalously high electrical conductivity in southern Scotland, known as the Eskdalemuir anomaly, has been discovered and mapped, in terms of related anomalies in time-varying geomagnetic disturbance fields^{1,2}. Magnetotelluric soundings³ have indicated the depth of the conductor as 12 km, at which depth Jacob⁴ has reported an anomaly in seismic wave velocities. Bamford and Prodehl⁵ have also found anomalous seismic wave velocities in this region. In 1973 the University of Edinburgh operated two magnetometer arrays which together covered nearly all of Scotland. In each array 20 three-component recording magnetometers of Gough-Reitzel type⁶ were operated for 6-8 weeks. These arrays, together with extended magnetotelluric study of the Eskdalemuir anomaly⁷, now make possible a fuller description and interpretation of conductive structures. Data from stations north of the Midland Valley, where only limited geomagnetic variation studies^{8,9} had preceded ours, indicate further regions of anomalously high conductivity in Scotland. Full discussion and analysis of these data will be presented elsewhere. This note gives some preliminary indications of these conductivity anomalies and suggests possible tectonic implications.

Time-varying magnetic fields associated with induced currents in the solid earth, which may delineate conductive structures, are superposed on the source field due to currents in the magnetosphere and ionosphere. At the geomagnetic latitude of Scotland,

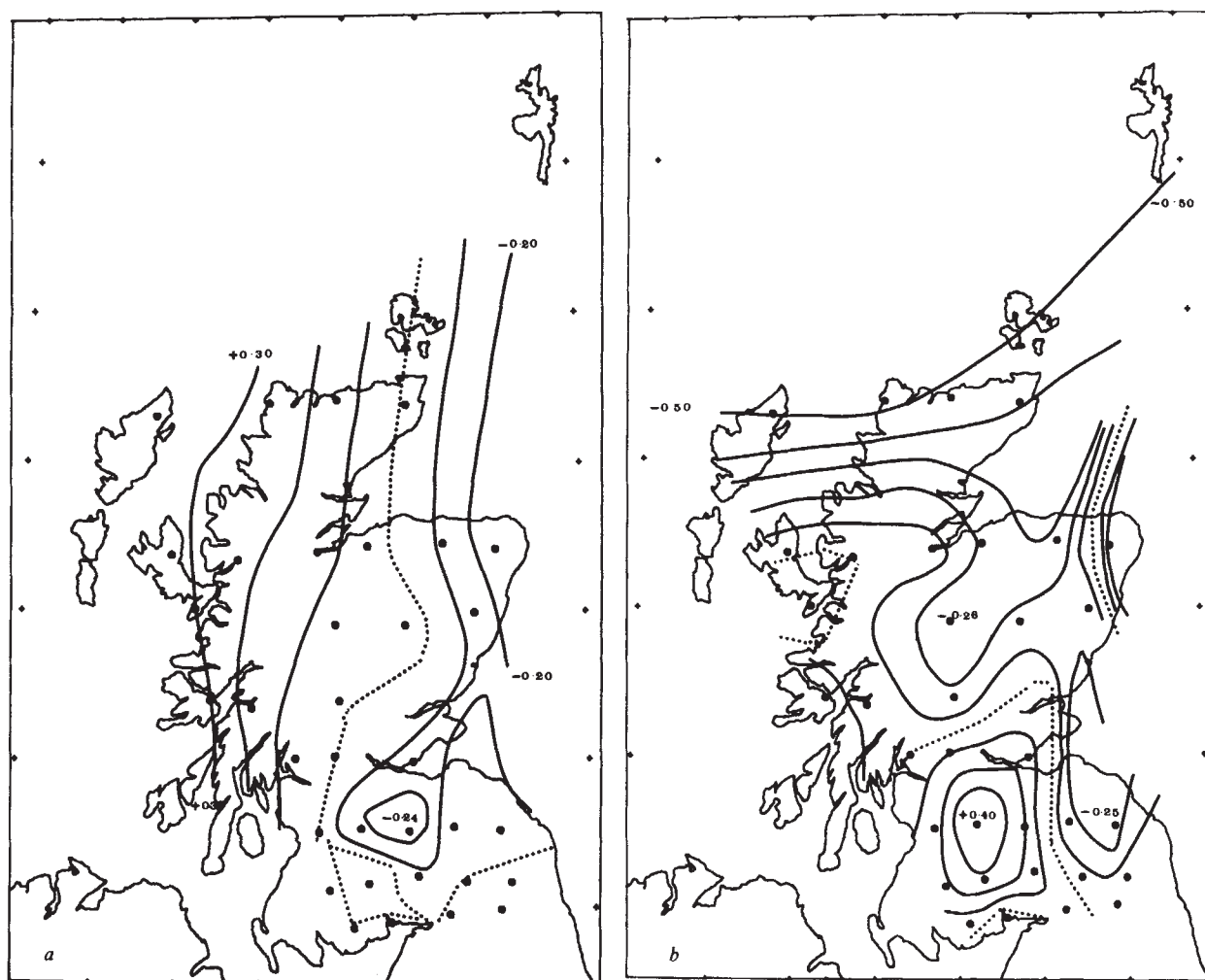
source fields associated with polar electrojet or substorm activity have complicated morphologies, which may make the recognition of internal anomalies difficult. Induction in the Atlantic Ocean and the seas surrounding the British Isles provides an additional complication, particularly at the longer periods. Several techniques have been applied to the interpretation of the very large body of data from the arrays. These include the contouring of amplitudes and phases of Fourier transforms and the mapping of polarisation ellipses and of in-phase and quadrature-phase induction arrows. These procedures have been less successful in Scotland than elsewhere¹⁰⁻¹³, presumably because of the superposition of spatially variable fields associated with the source and with the ocean on a complex geoelectric structure in northern Scotland.

One method which has proved useful in indicating the internal conductive structure is that of the hypothetical event¹⁴, in which contoured maps of the vertical magnetic field component, Z , are found for a hypothetical uniform horizontal inducing field in any desired azimuth. If the vertical field can be represented by a linear combination of the horizontal fields, then the transfer functions $A(f)$ and $B(f)$, at the frequency f of the field can be calculated from $Z = A(f)\bar{X} + B(f)\bar{Y} + \epsilon(f)$. Here $\bar{X}$ and $\bar{Y}$ are the Fourier transforms at frequency f of the northward and eastward components of the field and $\epsilon(f)$ is a small uncorrelated residual. By using appropriate values of $\bar{X}$ and $\bar{Y}$ to represent a desired hypothetical field and assuming that $\epsilon(f)$ is negligible, one can

obtain the Z response to the synthetic field using this equation and the estimated values of $A(f)$ and $B(f)$. The transfer functions are estimated from a number of variation events at each station¹⁵. While to some extent they reflect the relation of Z to X and Y in the source as well as in induced currents, the effect of the source on transfer functions derived from several events of differing source geometries and polarisations is smaller than that on a single event mapped, say, as Fourier transform coefficients. A second advantage of the hypothetical event method is that it substitutes for the natural source-fields, of complicated geometry and polarisation, a uniform, linearly polarised source field, the azimuth of which can be chosen to maximise the induced currents in a given structure. A third advantage is that this method facilitates unified interpretation of the results from the two consecutively-operated arrays.

Contoured maps of Z have been constructed for inducing fields linearly polarised in magnetic N, E, NW and NE azimuths, at periods in the range $300 \leq T \leq 5200$ s. For $T \geq 2400$ s the coastal and source field effects dominate the transfer functions in northern Scotland—as shown in Fig. 1(a) and (b)—but at shorter periods the internal currents predominate and produce a consistent and conspicuous pattern in the hypothetical-event Z contours in the range $300 \leq T \leq 1000$ s. Typical maps are shown in Fig. 2(a) and (b) for unit north-south inducing fields of periods 700 s and 1000 s. To a first approximation, a single current concentration produces closely spaced parallel contours in its vicinity. There may be a

Fig. 1 *a*, Z contours for a unit regional horizontal magnetic field directed eastwards. *b*, Z contours for a unit regional horizontal magnetic field directed northwards. In both cases, the values plotted are for the in-phase fields for a period $T=2400$ s, the contour interval is 0.10 nT and the zero Z contours are represented by the dotted curves.



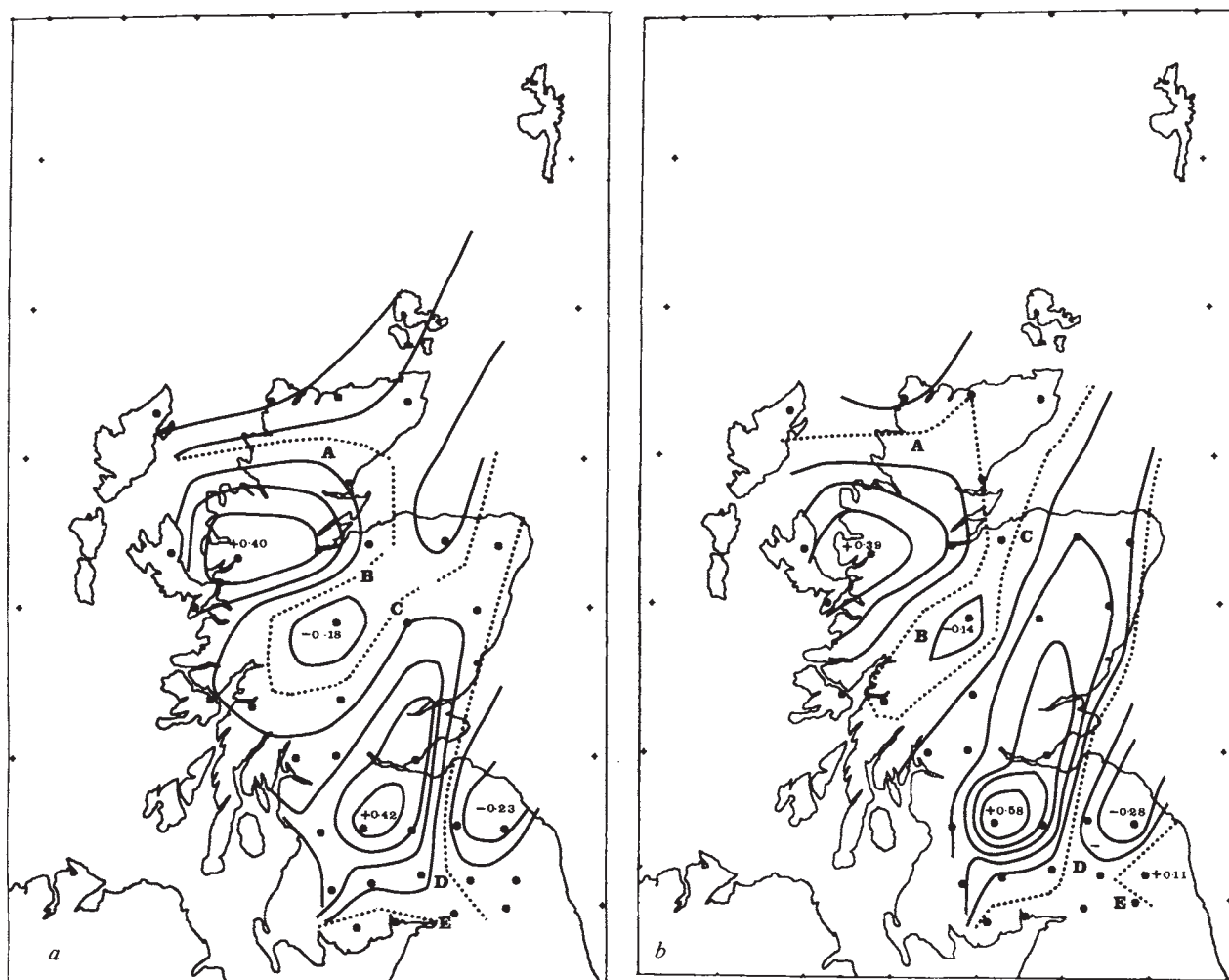


Fig. 2 Z contours for a unit regional horizontal field directed northwards. *a*, In-phase field for a period of 700 s. *b*, In-phase field for a period of 1000 s. The contour interval is 0.20 nT and the zero Z contours are represented by the dotted curves.

roughly central zero contour if there is no large regional gradient in Z due to other currents. In the maps the $Z = 0$ contours are delineated by dotted lines. For more complex current concentrations, the zero contours may not have the same significance. As shown by Bailey and Edwards¹⁶, the Z field gradients are more useful for interpretation in this case. The Eskdalemuir anomaly, which may be associated with two regions of zero Z contours (D and E), is prominent in contour maps at all periods. Three other regions of zero Z contours appear consistently at the shorter periods. The first (*a*) is in the north-west corner of Sutherland, the second (*b*) runs along the Great Glen and the third (*c*) follows a path in eastern Scotland from the Moray Firth to Argyll. The widths of the Z anomalies set maximum limits of about 35 km on the depths of all these conductors, so they are within the crust. The significance of the Z contours is now being studied further and should lead to quantitative modelling of the conductive structures and correlation with other geological and geophysical features. For the present, attention is drawn to the interesting correlation between the three zero Z regions A, B and C (Fig. 2) and two Moinean and one Dalradian ophiolite suites regarded by Garson and Plant¹⁷ as marking former subduction zones. These are indicated schematically in Fig. 3. These zones are associated with intense mineralisation—particularly the Dalradian zone which has, for example, well-defined outcrops of graphitic schists. The Moine belt of ophiolites, proposed by Garson and Plant as a late Precambrian subduction zone (750–800 Myr old), can be cor-

related in Sutherland with Z zero contour A and along the Great Glen with zero contour B. The third belt C seems to correlate with the Dalradian ophiolites, which Garson and Plant regard as marking a subduction zone active in Cambrian time. The three northern anomalous regions mapped by the magnetometer arrays may thus be related to these ancient subduction zones. It has been suggested that hydrated minerals in ophiolites may be highly conductive¹⁸ but in ancient subduction zones other sources of high electrical conductivity probably exist as suggested by Camfield and Gough¹⁹ for their proposed Proterozoic plate boundary in North America. Garson and Plant have also proposed two younger ophiolite suites along the Highland Boundary and S. Uplands faults but these are much less well defined and have less associated mineralisation. Many questions must be answered, however, before the association between the geomagnetic data and possible subduction zones can be made with confidence in Scotland. Further field observations, already in progress, should enable both the location and depth of the conductive zones to be located more precisely. Magnetotelluric measurements at locations traversing the anomalous regions in N. Scotland—planned to start in 1977—should indicate whether the Z contours should be associated with single or complex conductors. Recent magnetotelluric measurements in S. Scotland should assist in the interpretation of the regions D and E. Our results suggest a more complicated geometry for the Eskdalemuir anomaly than was shown by earlier work^{1,2}. There seem nevertheless sufficient

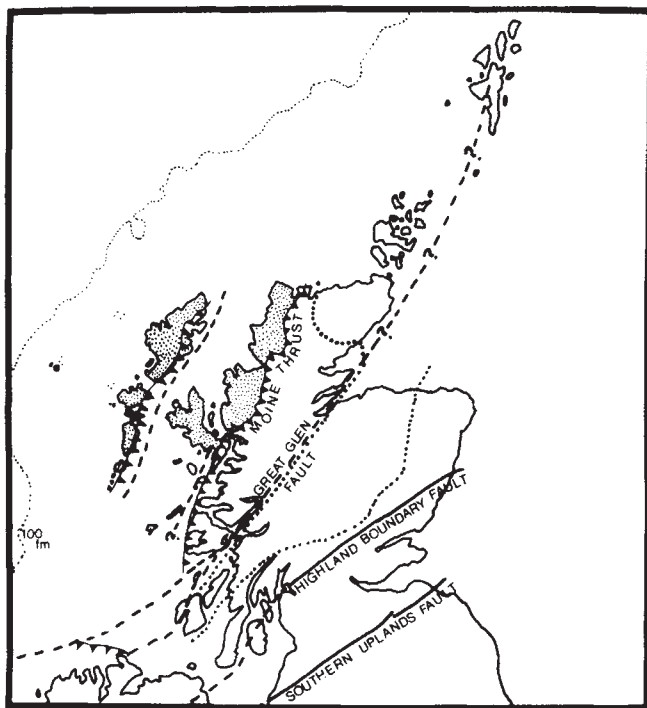


Fig. 3 Simplified tectonic map of Scotland. Shaded region is the Lewisian Foreland. Dotted curve represents ancient subduction zones proposed by Garson and Plant.

grounds to propose that there may be a close relation between the complex electrical structure of the crust in Scotland and past tectonic underthrusts.

We thank Mr C. Gold, Department of Geology, University of Alberta for a contouring programme for irregularly distributed data points. Financial support was provided by NERC and the NRC of Canada.

V. R. S. HUTTON
J. M. SIK

Department of Geophysics,
University of Edinburgh, UK

D. I. GOUGH

Institute of Earth and Planetary Physics,
University of Alberta, Canada

Received 15 December 1976; accepted 23 February 1977.

- ¹ Osimeikhian, J. E. A. & Everett, J. E. *Geophys. J. R. astr. Soc.* **15**, 361–366 (1968).
- ² Edwards, R. N., Law, L. K. & White, A. *Phil. Trans. R. Soc. A270*, 287–323 (1971).
- ³ Jain, S. & Wilson, C. D. V. *Geophys. J. R. astr. Soc.* **12**, 165–180 (1967).
- ⁴ Jacob, A. W. B. *Geophys. J. R. astr. Soc.* **18**, 189–197 (1969).
- ⁵ Bamford, D. & Prodehl, C. *J. Geol. Soc.* (in the press).
- ⁶ Gough, D. I. & Reitzel, J. S. *J. Geomagn. Geoelect.*, Kyoto **19**, 203–215 (1967).
- ⁷ Jones, A. G. & Hutton, V. R. S. *Acta geod. geophys. montan.* (in the press).
- ⁸ Booth, D. C. & Stuart, W. F. *Earth planet. Sci. Lett.* **22**, 413–416 (1974).
- ⁹ Green, C. A. *Geophys. J. R. astr. Soc.* **40**, 225–240 (1975).
- ¹⁰ Camfield, P. A., Gough, D. I. & Porath, H. *Geophys. J. R. astr. Soc.* **22**, 201–222 (1971).
- ¹¹ Gough, D. I., de Beer, J. H. & van Zijl, J. S. V. *Geophys. J. R. astr. Soc.* **34**, 421–433 (1973).
- ¹² Gough, D. I., McElhinny, M. W. & Lilley, F. E. M. *Geophys. J. R. astr. Soc.* **36**, 345–365 (1974).
- ¹³ Alabi, A. O., Camfield, P. A. & Gough, D. I. *Geophys. J. R. astr. Soc.* **43**, 815–834 (1975).
- ¹⁴ Bailey, R. C., Edwards, R. N., Garland, G. D., Kurtz, R. & Pitcher, D. *J. Geomagn. Geoelect.* **26**, 125–146 (1974).
- ¹⁵ Schmucker, U. *Anomalies of Geomagnetic Variations in the South-western United States* (University of California Press, 1970).
- ¹⁶ Bailey, R. C. & Edwards, R. N. *Geophys. J. R. astr. Soc.* **45**, 97–104 (1976).
- ¹⁷ Garson, M. S. & Plant, J. *Nature phys. Sci.* **242**, 34–38 (1973).
- ¹⁸ Hyndman, R. D. & Hyndman, D. W. *Earth planet. Sci. Lett.* **4**, 427–432 (1968).
- ¹⁹ Camfield, P. A. & Gough, D. I. *Can. J. Earth Sci.* (in the press).

Tectonic and sedimentary significance of Cretaceous Tekenika Beds of Tierra del Fuego

STRATA called Tekenika Beds have been considered the oldest post-orogenic rocks within the southernmost Andean Cordillera. Their significance in terms of regional tectonic history outweighs their small outcrop area on the east side of Hardy Peninsula on Hoste Island, Chile, about 160 km north-north-west of Cape Horn (Fig. 1). The Tekenika Beds, which are

2–3 km thick, are best known on Burleigh Peninsula on the south side of Tekenika Bay (Tekenika derives from two Yahgan Indian words *Teke uneka*¹), where they are largely sandstone and conglomerate (Figs 1 and 2). The basal contact has not been seen; however, the strata, although folded (ref. 2 and unpublished reports of Empresa Nacional del Petrole), are less deformed than the tightly folded Lower Cretaceous Yahgan Formation just north of Tekenika Bay less than 5 km away. Therefore an unconformity is implied. Coarseness of the strata, fossil wood (some bored by worms or molluscs), thin lignitic coal beds, shallow marine molluscs and presence of some plutonic clasts led to the view that the Tekenika is a nearshore, post-orogenic sequence². Suarez and Pettigrew recognise it as pre-orogenic, but still interpret it as a shallow marine deposit³. We report here structural evidence indicating that the Tekenika Beds are pre-orogenic and deep water deposits.

Dating of the Tekenika rocks has long seemed important in constraining the younger age limit of Andean orogenesis in Tierra del Fuego. For this reason, Halpern in 1963 collected fossils and igneous pebbles from the Burleigh Peninsula for isotopic dating. His results suggested a very equivocal palaeontological age of 'Late Cretaceous to Early Tertiary', and K–Ar dating of a gabbroic boulder suggested 116 ± 4 Myr (Early Cretaceous) as a maximum possible age². An altered greenstone considered contemporaneous with the lower Yahgan was dated as 92 ± 4 Myr, which was interpreted as being close to the time of folding and metamorphism². The gabbro clasts were considered derived from the Andean batholith complex just to the south-west of Tekenika Bay. Intrusives into the folded and cleaved Early Cretaceous (Neocomian) Yahgan Formation at two localities 50 km north on Hoste and Navarino Islands were also dated as 77 ± 5 – 81 ± 5 Myr (ref. 2).

The above evidence seemed consistent with the view that the Tekenika Beds represent a post-orogenic molasse. Because neither fossils nor isotopic dates could resolve satisfactorily exactly when the main Andean orogeny occurred between 116 and 77–81 Myr we visited, on a cruise of R. V. Hero, in May–June 1974, all the Tekenika outcrops, hoping to clarify stratigraphic relationships and discover more definitive fossils. Some fossils were found, but the only one of possible stratigraphic significance is a colonial coral fragment from conglomerate on Navidad Bay (Fig. 1). It was identified by A. G. Coates, George Washington University, as *Actinastrea* cf. *A. peruviana*, which is known from upper Aptian–to Albian-aged strata in Peru⁴. We also found *Rotalaria*, *Yoldia*?, a naticiform snail, belemnoids and *Ostrea* fragments. All of the shells and plants were contemporaneous with Tekenika sedimentation, but were swept in like pebbles. All that any of the palaeontological evidence clearly shows is that the strata are Cretaceous.

Having assumed that the Tekenika Beds post-dated the main Andean orogeny in Tierra del Fuego, we were surprised to discover structural relationships that show them to be pre-orogenic instead; that is, late Early Cretaceous to middle Late Cretaceous or post-Yahgan and pre-77–81 Myr. To summarise, Yahgan strata contain Neocomian fossils and lie below the Tekenika beds; the latter contain 116-Myr-old pebbles and a possible Albian–Aptian fossil. Folds and cleavage in the Yahgan are cut by 77–81-Myr plutons, and concordant cleavage was found in the Tekenika. Therefore, the Tekenika Beds are post-Neocomian and pre-77–81 Myr, possibly Albian or Aptian. The Tekenika Beds lie within a synclinal whose axis trends west-north-west-parallel to more tightly compressed folds in the Yahgan Formation north of Tekenika Bay (Fig. 1). North of Burleigh Peninsula we found alternating sandstones and olive-drab-weathering mudstones concordant with darker, more indurated graywackes and mudstones of the Yahgan Formation (Fig. 2). The basal contact of the sequence could not be located precisely because of intrusive dykes and poor exposures. Similar strata occur south-east of Burleigh Peninsula along Navidad Bay, but relationships of these lower Tekenika Beds to fossiliferous Yahgan strata (or Hardy Formation of

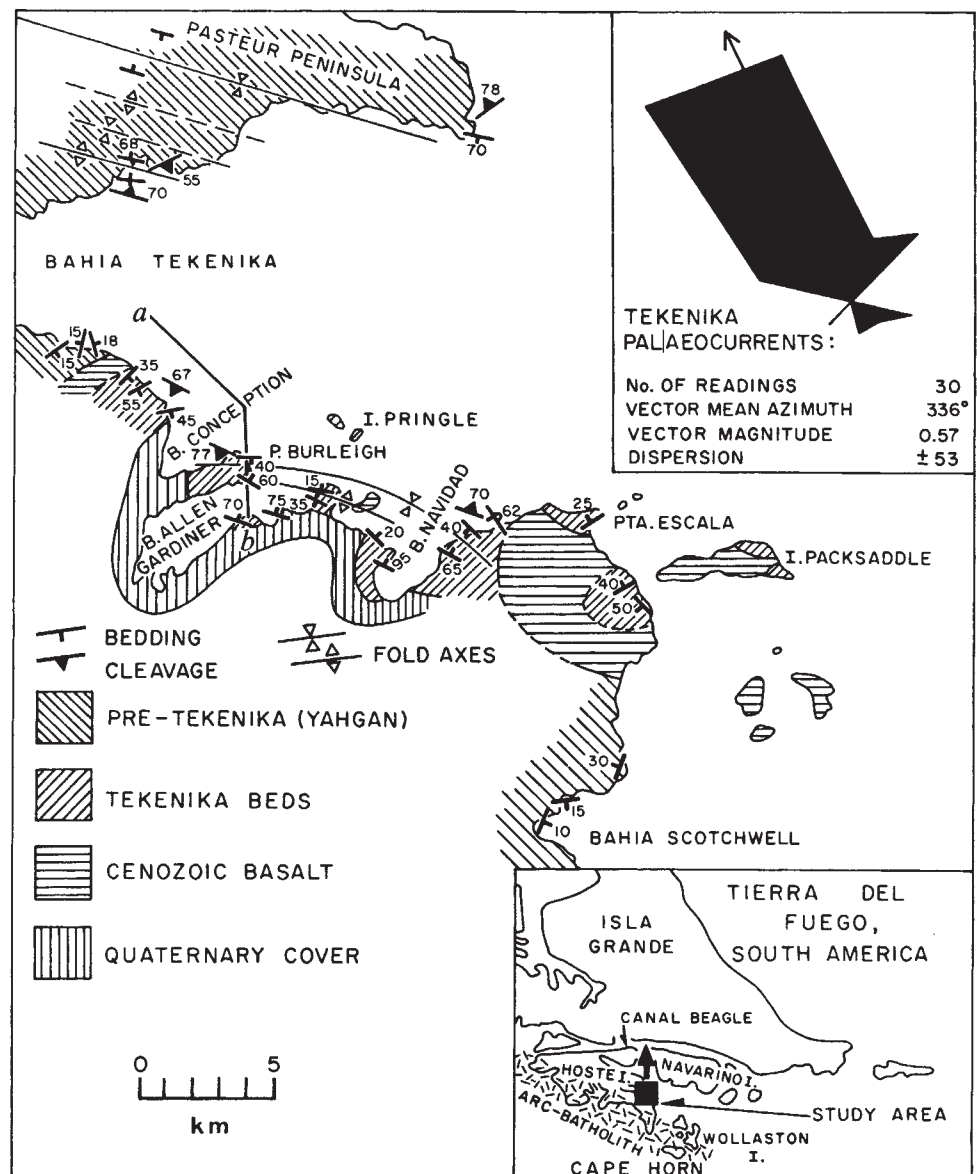


Fig. 1 Schematic geological map of Tekenika Bay area, southern Chile; pre-Tekenika at Bahia Scotchwell includes Hardy Formation of Suarez and Pettigrew^{3,8}. Heavy arrow in inset is average Yahgan palaeocurrent direction.

Suarez and Pettigrew³) farther south at Scotchwell Bay are obscured by a late Cainozoic basal tcomplex on Punta Escala and Isla Packsaddle (Fig. 1). Our observation of a subtle but definite cleavage in the Tekenika mudstones is particularly significant. Generally the cleavage consists of sporadically developed fractures, but locally it is slaty and parallels the regional slaty cleavage characteristic of the Yahgan rocks to the north.

Throughout this part of the southern Andes, deformation and metamorphism are remarkably heterogeneous, generally diminishing in intensity southwards from a maximum along the north side of Canal Beagle⁵⁻⁷. Just north of Tekenika Bay, the Yahgan rocks are tightly to openly folded, with a pronounced slaty cleavage. South of Tekenika Bay, however, at Bahia Scotchwell, for example, folding in the fossiliferous Lower Cretaceous rocks is gentle. The rocks here have a well developed joint system, but slaty cleavage is non-existent. Thus the slight deformation of the Tekenika rocks is consistent with the regional structure. Their less deformed and metamorphosed nature is not the result of a post-orogenic age, as long assumed, but reflects its structural location. We infer that the Tekenika Beds were originally more widespread, and have been preserved due to their location in the axis of a regional synclinorium.

Sedimentologically we also found much similarity with underlying Yahgan flysch strata. Both show the same south-

north palaeocurrent pattern (Fig. 1) and both are characterised by graded bedding throughout (Fig. 3); sandstones show typical Bouma sequences in both cases. The Tekenika grades upward from Yahgan-like alternating graywackes and mudstones into thick, crudely graded sandstones with less mudstone, and in turn graded and massive volcanic cobble and boulder conglomerates with some clast imbrication (Fig. 2); sandstones are poorly sorted lithic (volcanic) graywackes with considerable matrix^{2,11}. The coarse volcanoclastic deposits have sedimentary structures identical with those of conspicuous andesitic breccias and coarse sandstone zones interstratified within the generally finer Yahgan on western Navarino Island (Fig. 1). Both are generally poorly sorted and show massive as well as graded units. Parallel stratification is very common and some small- and medium-scale cross bedding is present in both cases. Shallow scour structures are present, especially in the Tekenika. Fragments in the Yahgan breccias are much less rounded than in the Tekenika conglomerates, however. Both cases lack abundant medium-scale cross bedding in well-sorted sands, pronounced channelling, fining-upward sequences, extensive organic burrowing of mudstones and *in situ* coals, all characteristic of deltaic deposits. Also lacking are low-angle inclined stratification in well-sorted sands, layers with shell or heavy mineral concentrations and soil profiles characteristic of littoral deposits.

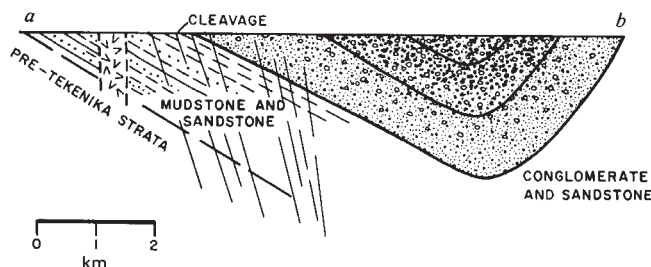


Fig. 2 Cross section of Tekenika Beds along line *a-b* shown in Fig. 1. Note cleavage and a dyke (left) penetrating both pre-Tekenika and Tekenika strata.

The Yahgan-aged (Neocomian) rocks coarsen southward toward the main Patagonian batholith; they have been named the Hardy Formation^{3,8}. Although Suarez and Pettigrew included the Tekenika Beds in their Hardy formation, we consider the former a member of the Yahgan formation because it shares deeper-water characteristics, structural concordance and grade of metamorphism with the Yahgan. Sedimentology of the Hardy rocks and their relationships to the Tekenika are not yet well documented. Just south of Scotchwell Bay are pillow lavas and volcanic breccias interstratified with Yahgan-like mudstones, sandstones and volcanic conglomerates containing marine Lower Cretaceous fossils. Near the southern end of Hardy Peninsula (inland from Orange Bay) we observed apparently non-marine silicic breccias with accretion lapilli and welded tuff zones interstratified with mafic and intermediate breccias and vesicular flows. The same assemblage occurs also on the Wollaston Islands 100 km to the south-east (Fig. 1). These volcanic rocks are of uncertain age, but the simplest hypothesis is that they comprise part of the Early Cretaceous volcanic arc complex. Cross-bedding orientations within coarse green sandstones indicate south-north palaeocurrents, which agree with those of the Yahgan and Tekenika strata.

Fig. 3 Graded Tekenika conglomerate with large ripped up mudstone block at base.



There are two laterally-equivalent Cretaceous sedimentary facies in southern Tierra del Fuego, which were both derived from an arc source—the generally finer Yahgan (north) and the coarser conglomerates and breccias (south)⁹. This situation is duplicated in equivalent rocks of South Georgia Island now 2,000 km to the east, but which is interpreted as originally having been adjacent to Navarino Island⁵. Significantly, the structural situation on South Georgia and Annekov Island is also similar to that in Tierra del Fuego, in that mid-Cretaceous deformation was concentrated towards the north, while sedimentary and igneous rocks deposited in and immediately behind the volcanic arc are only gently deformed^{5-7,10,11}.

We interpret the Tekenika Beds to be late Early to early Late Cretaceous in age, and to represent the youngest recognisable part of a continuous coarsening-upward (and southward) volcanoclastic succession. The typical Yahgan flysch to the north comprises submarine fan deposits debouched into a relatively deep, narrow marginal or back arc basin⁹⁻¹¹. We believe the Tekenika was deposited in a similar setting, but the coarse, upper Tekenika must represent an upper fan channel or suprafan lobe closer to the volcanic arc source than most of the Yahgan. The heterogeneous breccias, tuffs and flow rocks of southern Hoste and the Wollaston Islands (Hardy Formation) presumably were deposited within the arc itself; the batholiths are the roots of the arc^{5-8,11}. The Tekenika conglomerates and sandstones were derived from rocks such as these, and were redeposited below wave base rather than on deltas and shorelines. This is based on the prevalence of graded bedding and the fact that all of the plant and invertebrate material has clearly been transported. Land-derived logs up to 4 m long and oriented by currents as well as shallow-marine shells occur within conglomerates and coarse sandstones; the lignitic coal occurs as lenticular seams only a few cm thick at the tops of graded units. Rounding of the Tekenika gravel indicates derivation from beach or stream gravels formed originally on volcanic islands. Rare chaotic pebbly mudstones (diamictites) indicate mass gravity flows, while the graded conglomerates of both the Yahgan and Tekenika indicate transport by less viscous turbidity currents, debris flows or other gravity-driven mechanisms. Such resedimented conglomerates have received considerable study in recent years, and are proving to be rather common in rock sequences like those of the southernmost Andes.

We thank the crew of R. V. Hero, NSF (who financed the work), the Chilean Navy and Empresa Nacional del Petroleo. The manuscript was read by Dr M Halpern.

R. H. DOTT, JR
R. D. WINN, JR

Department of Geology and Geophysics,
University of Wisconsin,
Madison, Wisconsin 53706

M. J. DEWIT
R. L. BRUHN

Lamont-Doherty Geological Observatory,
Columbia University,
Palisades, New York 10964

Received 16 August 1976; accepted 5 January 1977.

- 1 Bridges, E. L. *Uttermost Part of the Earth*, 4th edn, 558 (Hodder and Stoughton, London, 1963).
- 2 Halpern, M. & Rex, D. C. *Bull. geol. Soc. Am.* **83**, 1881–1886 (1972).
- 3 Suarez, M. & Pettigrew, T. H. *Geol. Mag.* **113**, 305–400 (1976).
- 4 Iddings, A. & Olson, A. A. *Bull. Am. Ass. Petrol. Geol.* **12**, 1–39 (1928).
- 5 Dalziel, I. W. D., Dott, R. H., Jr, Winn, R. D., Jr & Bruhn, R. L. *Bull. Geol. Soc. Am.* **86**, 1034–1040 (1975).
- 6 Bruhn, R. L. dissertation, Columbia Univ., 60 (1976).
- 7 Bruhn, R. L. & Dalziel, I. W. D. in *Maurice Ewing, Series I, Island Arcs, Deep Sea Trenches and Back-Arc Basins* (eds Talwani, M. & Pitman III, W. C.) (Am. Geophys. Un., in the press).
- 8 Suarez, M. *Geology* **4**, 211–214 (1976).
- 9 Winn, R. D., Jr, & Dott, R. H., Jr. *A Mtg Abstr., Am. Ass. petrol. geol. and Soc. Econ. Paleont. Miner.* **82** (1975).
- 10 Winn, R. D., Jr, dissertation, Univ. Wisconsin (1975).
- 11 Winn, R. D., Jr & Dott, R. H. *Submarine Canyon and Fan Sedimentation* (eds Stanley, D. J. & Kelling, G.) (Dowden, Hutchinson & Ross, in the press); *A Meeting Abstr. Am. Ass. petrol. Geol.* **127** (1976).

Rapid removal of plutonium from the oceanic surface layer by zooplankton faecal pellets

It is possible that increasing quantities of plutonium will be introduced into the marine environment, so detailed knowledge of the oceanic behaviour of this element is highly desirable. The levels of plutonium in sea water and marine organisms have been established in a general way^{1,2}, and suggestions have been made as to possible transport mechanisms. Noshkin and Bowen³ proposed a model which associates plutonium with a mixed population of particles, sinking at rates of 70–392 m yr⁻¹, and Folsom *et al.*⁴ suggested that algae and, perhaps, the main phytoplanktonic biomass may have an important role in determining the chemical and physical forms of plutonium predominant in the ocean. The role of the zooplanktonic biomass has not been investigated in detail, but it is known that for several elements zooplankton metabolism may be an important biological factor in the removal of the elements from the surface layer of the ocean to depth^{5–9}. The particular importance of zooplankton faecal pellets in this process has been highlighted, and the faecal pellets of a common zooplanktonic species, the euphausiid *Meganyctiphanes norvegica*, have been shown to be rich in the important naturally-occurring alpha-emitter ²¹⁰Po when compared with whole organism levels¹⁰. We have performed a similar study for plutonium, and we report here that *M. norvegica* faecal pellets are indeed relatively rich in plutonium, and suggest that zooplankton faecal pellet deposition might be an important vector in the vertical oceanic transport of the element.

The environmental levels of plutonium are extremely low, and collection of adequate sample material was a major problem; a faecal pellet collection system was devised¹¹ which provided us with faecal pellet samples of about 100 mg dry mass. Oven-dried samples were acid-digested, treated with hydrofluoric acid, and then subjected to previously described ion exchange techniques¹² to isolate plutonium; ²³⁶Pu was used as a spike. Thin sources were prepared and counted by α -spectroscopy. Exacting chemistry was necessary to achieve consistently low 'blank' counting rates, and long counting times were commonplace. The results are given in Table 1, together with the ²³⁹+²⁴⁰Pu concentrations in whole euphausiids, euphausiid exoskeletons collected by dissecting 336 euphausiids, and in a bulk sample of microplankton which serve as food for *M. norvegica*. We found that the concentrations in faecal pellets, although varying widely, were two orders of magnitude larger than those in whole euphausiids and an order of magnitude higher than in the typical food organisms; plutonium levels in the faecal pellets were in fact vastly in excess of those found in typical marine biological materials².

What are the implications of these high concentrations in faecal pellets for the flux of plutonium through living euphausiids? A plutonium atom ingested or absorbed by a living euphausiid can be eliminated from the animal by faecal pellet excretion, by moulting, by egg production or by soluble excretion; alternatively, it can be

Table 2 Flux rates for various transfer modes of plutonium through living euphausiids*

	²³⁹ + ²⁴⁰ Pu (pCi kg ⁻¹ d ⁻¹)
Faecal pellets	5.4
'Moult'†	0.011
Soluble excretion	0.032
Assimilation	0.006

* The flux rates listed in this table and discussed in the text represent three output fluxes plus the assimilation. The sum of these four terms must of course equal the input flux rate, which represents the plutonium taken up by the animal from both water and food. No information about the input via the water route is available, and at this stage only a crude estimate of the input from food can be made. This is done by multiplying the known food ingestion rates for *M. norvegica*, namely between 0.113 and 0.320 g food per g dry animal per day (ref. 9), by the plutonium concentration in microplankton given in Table 1. The result is a 'food only' input flux rate of between 0.6 and 1.6 pCi plutonium per kg per day, which is of the same order of magnitude but less than the estimated total output flux rate of over 5 pCi per kg per day. Fairly wide variation of element concentrations from one microplankton sample to another is, however, to be expected¹⁰; a more specific identification of the *M. norvegica* food as well as further determinations of the plutonium content of faecal pellets will be needed before the flux balance can be checked accurately and the relative importance of the food and water intake routes assessed.

† The flux via 'moult' quoted above is of course only an approximation, since the concentration in true moults may not necessarily be the same as in the exoskeletons which formed our sample. We have not yet been able to collect euphausiid moults in sufficient quantity to detect plutonium, but we hope to do so in the future; it is, however, very unlikely that the argument which we give in the text will be altered substantially.

assimilated during growth in one of the organs or tissues and remain in the animal. We calculate the flux of plutonium in a living euphausiid by using available biological data⁹ for *M. norvegica*. We multiply the typical faeces production rate, 0.038 g dry faeces per g dry animal per day, by the mean plutonium concentration in faecal pellets given in Table 1. We multiply the moulting rate, 0.009 g dry moult per g dry animal per day, by the concentration in the euphausiid exoskeleton. Collection of eggs in sufficient quantity is an unrealistic task; fortunately, the egg production rate^{9,10}, about 0.002 g dry eggs per g dry animal per day, is low, and trace element concentrations in eggs are usually unexceptional. It is extremely unlikely that this term will contribute significantly to the flux. To estimate the loss by soluble excretion we performed experiments of a type previously described for other elements¹⁰, using live euphausiids labelled with ²³⁶Pu, and found a soluble fractional excretion rate of 0.08 ± 0.02 per day. This value multiplied by the plutonium concentration in whole euphausiids gives the flux loss by soluble excretion. Finally, the amount of assimilated plutonium is obtained by multiplying the whole animal concentration by 0.015 g dry mass increase per g dry animal per day, an average growth rate for non-larval individuals.

The results of these flux calculations are given in Table 2. The complete dominance of the faecal pellet term is immediately obvious, and indicates that 99% of the plutonium taken up by *M. norvegica* is excreted in the faecal pellets. Euphausiid pellets sink fairly rapidly¹³, at rates ranging from 126 to 862 m d⁻¹, and they decompose relatively slowly⁸; it seems clear that they have the potential to reach the sea bottom in most areas. If the *M. norvegica*

Table 1 Concentrations of ²³⁹+²⁴⁰Pu in zooplanktonic material and microplankton

Sample	Wet-dry weight ratio	Individual determinations* pCi kg ⁻¹ dry	Mean pCi kg ⁻¹ dry
Whole euphausiids	4.7	0.32 ± 0.08; 0.47 ± 0.08	0.4
Euphausiid exoskeleton	4.3	1.2 ± 0.4	1.2
Euphausiid faecal pellets†	4.4	310 ± 50; 76 ± 8; 39 ± 14	142
Microplankton‡	10.7	4.9 ± 0.8	4.9

* Each individual determination is in fact the mean of two determinations made on the two halves of a split sample.

† High concentrations of certain trace elements^{9,18} and radionuclides¹⁹ in faecal material brought about by biological amplification processes have been noted previously. The fact that the plutonium concentration in the faecal pellets is higher than that in the food is simply a reflection of a high discrimination factor against a biologically non-essential element.

‡ Principally small crustacea, phytoplankton and detritus collected with 65-µm mesh size plankton net along with the euphausiids.

data can be considered as typical of the marine zooplankton biomass as a whole, we can make a rough estimate of the removal time¹⁰ of plutonium from the upper mixed layer of the ocean by zooplankton faecal pellets alone. We multiply the faecal pellet flux rate from Table 2 by $10^{-4} \text{ kg m}^{-3}$, a reasonable, if controversial, estimate^{10,14-16} of the dry zooplanktonic biomass in the upper mixed layer, and divide this product into 0.7 pCi m^{-3} , a typical value (ref. 2) of the $^{239+240}\text{Pu}$ concentration in surface sea water. The result is 3.6 yr, a figure which one would like to compare with estimates of the removal time of plutonium from the upper mixed layer by all routes. Unfortunately, direct quantitative estimates of this quantity are not available; the indications^{3,17} are, however, that it is short—of the order of 1 yr. Our estimate for the removal time due to zooplanktonic faecal pellets alone is of the same order, suggesting that faecal pellet removal has a significant role in the removal of plutonium from the surface layers of the sea. Investigations of possible competing removal routes (for example sinking phytoplankton detritus, large inorganic particulate settling) are needed to substantiate this suggestion.

This work was supported partially by the South African National Council for Oceanographic Research. We thank the Station Zoologique de Villefranche, France, for furnishing the necessary ship's time.

J. J. W. HIGGO
R. D. CHERRY

Physics Department,
University of Cape Town,
Rondebosch, C.P.,
South Africa

M. HEYRAUD
S. W. FOWLER

International Laboratory of Marine Radioactivity,
Musée Océanographique,
Principality of Monaco

Received 20 September 1976; accepted 2 February 1977.

- ¹ Noshkin, V. E. *Hlth Phys.* **22**, 537–549 (1972).
- ² Cherry, R. D. & Shannon, L. V. *Atom Energy Rev.* **12**, 3–45 (1974).
- ³ Noshkin, V. W. & Bowen, V. T. in *Radioactive Contamination of the Marine Environment*, 671–686 (IAEA Vienna, 1973).
- ⁴ Folsom, T. R., Hodge, V. F. & Gurney, M. E. *Mar. Sci. Commun.* **1**, 39–49 (1975).
- ⁵ Osterberg, C., Carey, A. G. & Curl, H. *Nature* **200**, 1276–1277 (1963).
- ⁶ Lowman, F. G., Rice, T. R. & Richards, F. A. in *Radioactivity in the Marine Environment*, 161–169 (National Academy of Sciences, Washington DC, 1971).
- ⁷ Fowler, S. W., Heyraud, M., Small, L. F. & Benayoun, G. *Mar. Biol.* **21**, 312–325 (1973).
- ⁸ Small, L. F. & Fowler, S. W. *Mar. Biol.* **18**, 284–290 (1973).
- ⁹ Small, L. F., Fowler, S. W. & Keckes, S. in *Radioactive Contamination of the Marine Environment*, 437–452 (IAEA, Vienna, 1973).
- ¹⁰ Cherry, R. D., Fowler, S. W., Beasley, T. M. & Heyraud, M. *Mar. Chem.* **3**, 105–110 (1975).
- ¹¹ La Rosa, J. *Deep Sea Res.* **23**, 995–997 (1976).
- ¹² Wong, K. M. *Analyt. Chim. Acta* **56**, 355–364 (1971).
- ¹³ Fowler, S. W. & Small, L. F. *Limnol. Oceanogr.* **17**, 293–296 (1972).
- ¹⁴ Mauchline, J. & Fisher, L. R. *Adv. mar. Biol.* **7**, 374–391 (1969).
- ¹⁵ Parsons, T. H. *Underwater J.* **4**, 30–37 (1972).
- ¹⁶ Jackson, P., *J. Cons. perm. int. Explor. Mer* **20**, 167–174 (1954).
- ¹⁷ Folsom, T. *Hlth Phys.* **29**, 597 & 611 (1975).
- ¹⁸ Boothe, P. N. & Knauer, G. A. *Limnol. Oceanogr.* **17**, 270–274 (1972).
- ¹⁹ Heyraud, M., Fowler, S. W., Beasley, T. M. & Cherry, R. D. *Mar. Biol.* **34**, 127–136 (1976).

Growth rate and longevity in *Drosophila melanogaster* and *Tribolium castaneum*

THERE are two main groups of hypotheses concerning the mechanism of ageing and death. The stochastic hypothesis assumes that ageing is due to the progressive deterioration of the metabolic machinery caused by the random accumulation of disturbances in one or the other of the cellular macromolecules. This hypothesis has been subject to many experimental tests, but is not yet supported by conclusive evidence. The developmental hypothesis of ageing considers senescence and death as the programmed ultimate stage of differentiation and development. Few experiments have been designed to test that last hypothesis. In two species of insects, *Drosophila* and *Tribolium*—classical tools in genetical research—we tried to verify the hypothesis which relates development to ageing. In *Drosophila* we looked at the variations of longevity linked to experimentally induced variations of one of the important components of development, namely growth rate. In *Tribolium*, we studied

variations in longevity associated with the natural variations of growth rate of various ecotypes. In both cases there are clear relations between pre-imaginal growth rate and adult longevity.

Ageing and death, at the level both of the cell and of the organism, are generally considered to be the consequences of a loss of molecular information. Information loss is considered as either the result of random events or as linked to development and therefore as the programmed endpoint of differentiation. Certain previsions of the modern stochastic hypothesis—the error-catastrophe theory¹—have been confirmed by experiment, but available evidence remains inconclusive^{2,3}. The developmental theory of ageing on the other hand is not easily tested by experiment. Yet as early as 1963, Muller⁴ stated that spontaneous ageing is part of normal development, that is, that development is a continuing process of which senescence forms the last stage or, in other words, that ageing is a built-in consequence of differentiation. From the latter considerations it has been deduced, correctly but probably incompletely, that the only likely way of prolonging lifespan involves a stretching of the developmental programme, essentially through a prolongation of immaturity⁵. Such a prolongation of total lifespan through a serious prolongation of immaturity, but at the sacrifice of part of the adult longevity, is not entirely unknown; it was repeatedly obtained, for instance in rats receiving various incomplete diets during adolescence^{6,7}. We may ask, however, how far does interference with the development and differentiation of an organism during adolescence have any paramount effect on the future and latest steps of life? Indeed at that stage of life, differentiation and development are almost complete. Interference during the earliest embryonic stages of life seems more promising. Poikilotherms thus seem more suitable than homeotherms for use in testing a programmed senescence theory of ageing, simply because the early developmental homeostatic mechanisms of poikilotherms are not strictly regulated, although there is good evidence for temperature acclimation.

The adult longevity of a single genotype of *D. melanogaster*, samples of which had been grown in a large array of pre-imaginal environments known to induce variations in both size and duration of development, was measured in a single controlled environment. The growth rate, estimated by the ratio of thoracic size to duration of development, was negatively and highly significantly correlated to longevity⁸ (Fig. 1a). In explaining the results the authors supposed that adult longevity is determined early in life by a defined programme. They further argued that this programme and growth rate could be concomitantly modified by varying the preadult environment. These findings apparently imply that within a species the variations in growth rate, characteristic of the various races of that species, should be reflected in the variations of the average longevities of those races. To test this deduction we studied the growth rate and adult longevity of various genotypes of *T. castaneum*—wild strains from wide geographic origin⁹—in a single controlled environment.

The environmental conditions were 33 °C, 70% relative humidity and standard medium of 95% whole wheat supplemented with 5% dried yeast. Growth rate was measured as the weight of the larva at a fixed time, the thirteenth day of the larval stage. Adult longevity was estimated during a maximum period of 227 d. Figure 1b shows the relationship between preimaginal growth rate and adult longevity of eight wild strains of *T. castaneum*. The variations in both traits are quite large and the relationship between them is positive and highly significant ($r=0.833$; $n=8$; $P<0.01$). It should be emphasised that there is no strong correlation between longevity and development time ($r=0.569$; n.s.) or adult weight ($r=-0.188$; n.s.) (ref. 9).

Thus in *Drosophila* an artificially obtained decrease in growth rate of a single hybrid genotype results in an increase

in adult longevity, whereas in *Tribolium* the natural variations in growth rate of various wild genotypes are positively correlated with longevity. Except for those differences in experimental approach *Tribolium* and *Drosophila* differ at least in three major ways directly related to growth rate and ageing. First, duration of development in *Tribolium* seems to be under polygenic control¹⁰, whereas in *Drosophila* selection for fast or slow development is generally unsuccessful¹¹. Second, the imaginal longevity of both species are quite different; in optimal conditions the average longevity of *Drosophila* is about 50 d but in *Tribolium* it is rather more than 130 d. Third, and most importantly, in contrast to *Drosophila*¹², somatic cell division apparently occurs during the imaginal stages of the life cycle of *Tribolium*¹³.

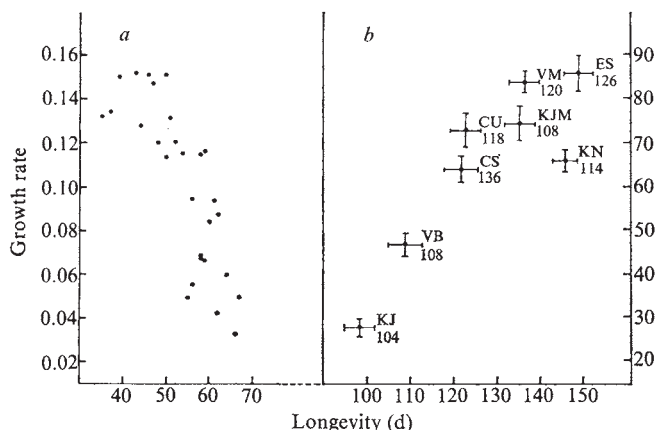


Fig. 1 *a*, Adult longevity as a function of growth rate in *Drosophila* ($r = -0.801$; $n = 28$; $P < 0.001$). Growth rate is estimated as the ratio between the cube of the thoracic size and the duration of development. Longevity is measured at 25 °C on 14 samples of two reciprocal hybrids raised in various pre-imaginal environments—six different temperatures, eight different pre-imaginal population densities—which are known to modify both the size and the duration of development of the treated individuals (redrawn from ref. 8). *b*, Adult longevity $\pm$ standard error plotted against growth rate, measured as mean 13-d larval weight, of eight wild strains of *T. castaneum*. The strains are identified according to their geographic origin as: CS (Capetown, South Africa), CU (Chicago, USA), ES (Edinburgh, UK), KJ (Kyoto, Japan), KJM (Kingston, Jamaica), KN (Kano, Nigeria), VB (Vicosia, Brazil) and VM (Veracruz, Mexico). Longevity measurement is based on observing adult death every 10 d up to a maximum of 227 d from egg. This period covered most of the adult death for all strains and the percentage of adults still alive after this period (with a maximum of 18% for ES) is highly correlated with the longevity estimates ($r = 0.840$; $n = 8$; $P < 0.01$). Numbers indicate the sample size of each strain.

In *Drosophila* longevity thus seems to depend negatively on growth rate. Growth rate is the productivity per unit of time, the resultant, due to genotype-environment interactions, of anabolism and catabolism. Growth rate may be associated with mitotic division rate. When growth rate is accelerated or decelerated it may, as a first approximation, be admitted that the same happens for mitotic division rate. Bozcuk¹² has demonstrated that the tissues of *Drosophila* show no mitoses after emergence of the imago, so the death of the organism must, to a certain extent, coincide with cellular death, or at least with the death of a certain number of cells.

It is known from studies carried out *in vivo* in higher organisms that, when a cell enters the ageing pathway, it has a life expectancy which is characteristic of the tissue¹⁴. When the mitotic rate of the tissue is high, as in the intestinal lining, the life expectancy of the functional cell is only a few days; when it is moderate, as in epidermis, the life expectancy may be a few weeks; and when it is low, as in liver, the life expectancy may exceed 1 yr. Furthermore, artificial prolongation of the epidermal cell life, and of the

liver cell life, may be obtained in conditions of stress which provoke a decreased mitotic rate¹⁴. These relationships between growth rate, mitotic division rate and longevity do not necessarily demonstrate the validity of the developmental theory of ageing, nor do they exclude the error-catastrophe theory. On the one hand, in relation to the developmental theory it may be supposed (as was done to explain the influence of preimaginal temperature on adult life-span¹⁵) that the growth rate affects the type of molecules that are synthesised and the rates at which these molecules carry out metabolism. If the idea of morphogenesis¹⁶ is accepted, it may then be supposed that variations in the type of molecules present in a cell affect the course of differentiation and development. Variations in the molecular composition of a cell as a function of growth rate are not unknown; in *Escherichia coli* for instance the protein composition of the ribosomes varies as a function of growth rate¹⁷. A changing growth rate could thus result in a different cytoplasmic molecular composition or configuration which could explain an earlier or later programmed death of the cells and of the organism. On the other hand, if a slower mitotic rate is accompanied by a lower number of errors of replication (a point which as yet has not been demonstrated) this could explain a later, randomly provoked unprogrammed cellular and organismic death.

In *Tribolium*, where mitotic divisions do not cease after emergence¹³, growth rate is, in contrast to *Drosophila*, positively correlated with adult longevity. So it seems that a faster growth rate, resulting probably in a shorter cellular life expectancy, must be associated in a certain number of stem cells with a higher potential number of cell divisions. The death of the individual will not coincide with the death of some particular cells but with the end of mitotic divisions in particular stem cells. Although the problem has been studied, little is known about the *in vivo* limited or unlimited lifespan of particular stem cells¹⁸. One may thus hypothesise that a modification in mitotic rate, through a modification of an asymmetrical type of mitotic division¹⁹, may result in a modified cytoplasmic molecular composition or configuration, with possible implications on cellular fate.

F. A. LINTS
M. H. SOLIMAN

Laboratoire de Génétique,
Université de Louvain,
1348 Louvain-la-Neuve, Belgium

Received 19 October 1976; accepted 15 February 1977.

- Orgel, L. E. *Proc. natn. Acad. Sci. U.S.A.* **49**, 412–416 (1963).
- Holliday, R. *Gerontologia* **21**, 64–68 (1975).
- Gershon, D. & Gershon, H. *Gerontologia* **22**, 212–219 (1976).
- Muller, H. J. in *Cellular Basis of Aetiology of Late Somatic Effects in Ionising Radiations* (ed. Harris, R. J. C.) (Academic, New York, 1963).
- Comfort, A. *Nature* **217**, 320–322 (1968).
- MacCay, C. M., Maynard, L. A., Sperling, G. & Barnes, L. L. *J. Nutr.* **18**, 1–13 (1939).
- Osborne, T. O., Mendel, L. B. & Ferry, E. L. *Science* **45**, 20–21 (1917).
- Lints, F. A. & Lints, C. V. *Expl. Geront.* **6**, 427–445 (1971).
- Soliman, M. H. & Lints, F. A. *Gerontologia* **21**, 102–116 (1975).
- Englert, D. C. & Bell, A. E. *Genetics* **64**, 541–552 (1970).
- Lints, F. A. & Gruwez, G. *Mech. Ageing Dev.* **1**, 285–297 (1972).
- Bozcuk, A. *N. Expl. Geront.* **7**, 147–156 (1972).
- Devi, A., Lemonde, A., Srivastava, V. & Sarkar, N. *Expl. Cell. Res.* **29**, 443–450 (1963).
- Bullough, W. S. *The Evolution of Differentiation* (Academic, New York, 1967).
- Burcombe, J. V. & Hollingsworth, M. J. *Gerontologia* **16**, 172–181 (1970).
- Crick, F. *Nature* **225**, 420–422 (1970).
- Deusser, E. & Wirtmann, H. G. *Nature* **238**, 269–270 (1972).
- Reincke, V., Burlington, H., Cronkite, E. P. & Laissve, J. in *Biology of Aging and Development* (ed. Thorbecke, G. J.) (Plenum, New York and London, 1975).
- Sheldrake, A. R. *Nature* **250**, 381–385 (1974).

Albedo measurement for remote sensing of crop yields

ANY remote sensing programme designed to estimate regional agricultural productivity has three major requirements. First, the crops present must be identified; second, the amount of land planted to each type must be determined, and third, the

yields of the crops per unit land area must be evaluated. The large area crop inventory experiment (LACIE) uses measurements of reflected solar radiation to fulfil the first two requirements in a true remote sensing context, but to establish yield, LACIE relies on statistical and climatological models largely dependent on ground-based observations¹. Here we report the basis for a crop yield prediction programme that is not subject to this restriction. Working with wheat, we have shown that the intensity of reflected solar radiation can be used to evaluate crop yields per unit land area. Linked to a satellite system, this could make the worldwide surveillance of agricultural production a technological reality that could greatly stabilise erratic fluctuations in world grain trade of the type which have recently disturbed the world economy.

On 3 December 1975, a 72 × 90-m field of Avondale loam that had been given an initial application of nitrogen fertiliser was planted with spring wheat (*Triticum durum* Desf. variety Produra). The field was then divided into six nearly equal north-south oriented rectangular plots. Five days after planting, each plot was irrigated with 10–12 cm of water. There was no further irrigation until 83 d after planting, when a programme was started to develop six soil water content regimes, as shown in Fig. 1. Each irrigation usually supplied about 10–12 cm of water. The few rains supplied a total of 4.7 cm.

The albedo data plotted on Fig. 1 were obtained from measurements of incoming and reflected solar radiation over each plot, as determined by one upright and six inverted Eppley pyranometers located about 50 cm above the top of the crop canopy. The data were recorded every 20 min, summed to give daily totals to determine mean albedo values, and then normalised as described before² to remove variability caused

by the changing altitude angle of the Sun during the growing season.

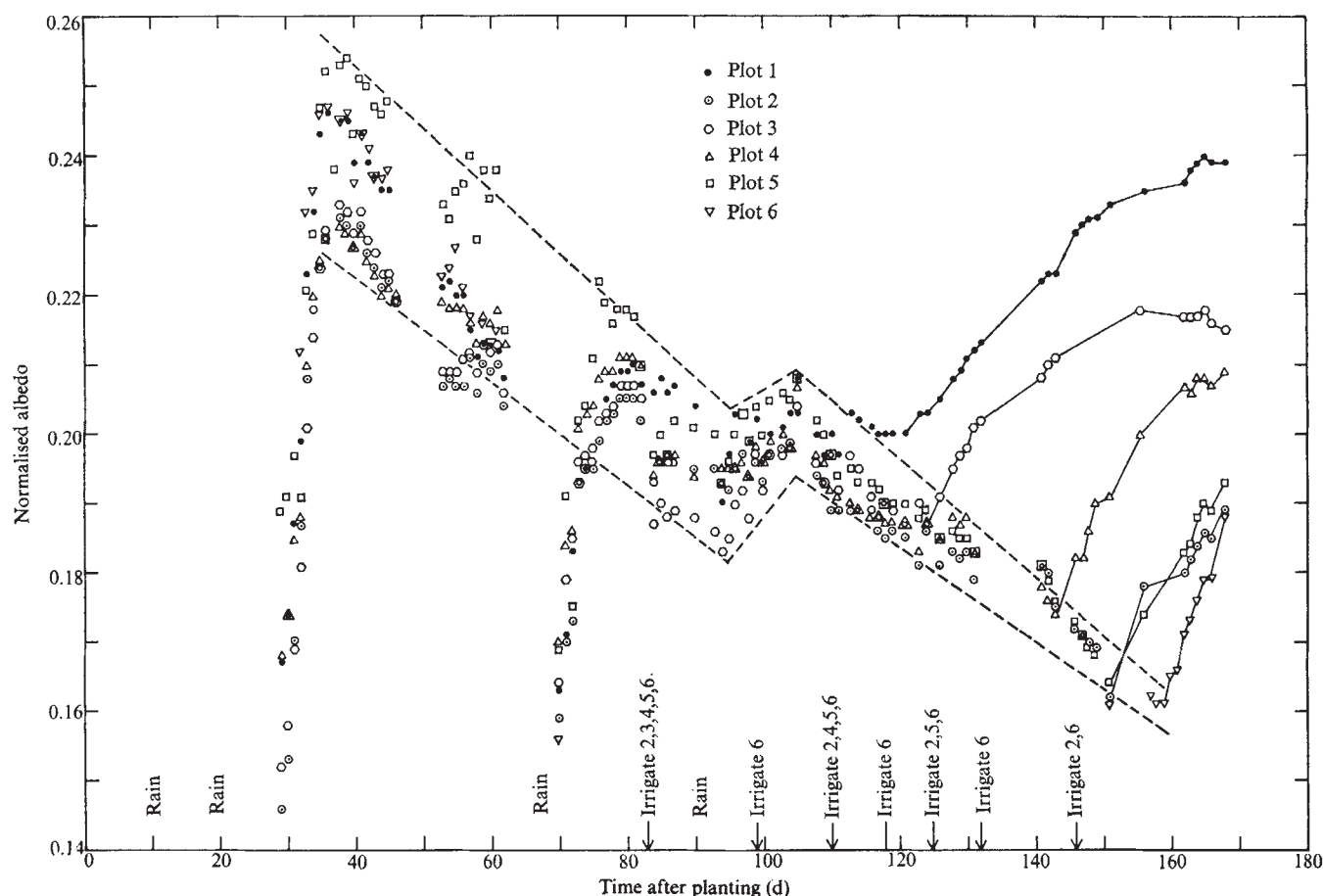
Twice weekly, green leaf area index (the ratio of green leaf area to the area of soil supporting its growth) was measured on three randomly selected plants per plot with an optically integrating area meter. When the heads emerged, three other randomly selected plants from each plot were sampled to determine mean head weight after driving off moisture by drying in an oven. To express these data per unit soil area, as in Fig. 2, at the end of the growing season we hand-harvested wheat from 14-m² sections of each of the six plots and counted every plant to obtain the number per unit soil area.

Figure 1 reveals a decline in albedo as the season progresses. Figure 2 shows that this is due initially to the growth and proliferation of the leaves (as they progressively shut out more and more of the high-albedo soil from view) and finally to the growth and proliferation of the plant heads (which start about day 100, when green leaf area has either reached a maximum or begun to decrease).

These relationships are shown more clearly in Fig. 3, where albedo data are first plotted as a function of green leaf area index until day 95, and then as a function of head dry weight from day 105 to near the end of head growth (when final increases in albedo began). The strong linear relation supports the idea that final grain yield is a linear function of the minimum albedo reached just before the large increases that occur as the heads begin to ripen. Comparison of these two parameters (Fig. 4) suggests that the hypothesis is verified by our data.

This analysis shows that measurements of reflected solar radiation can be used to estimate agricultural productivity in a complete remote sensing context. Our approach makes use of ground-based measurements of incoming solar radiation

Fig. 1 Clear-day normalised albedo of six differentially-irrigated plots of Produra wheat grown at Phoenix, Arizona as a function of days after planting.



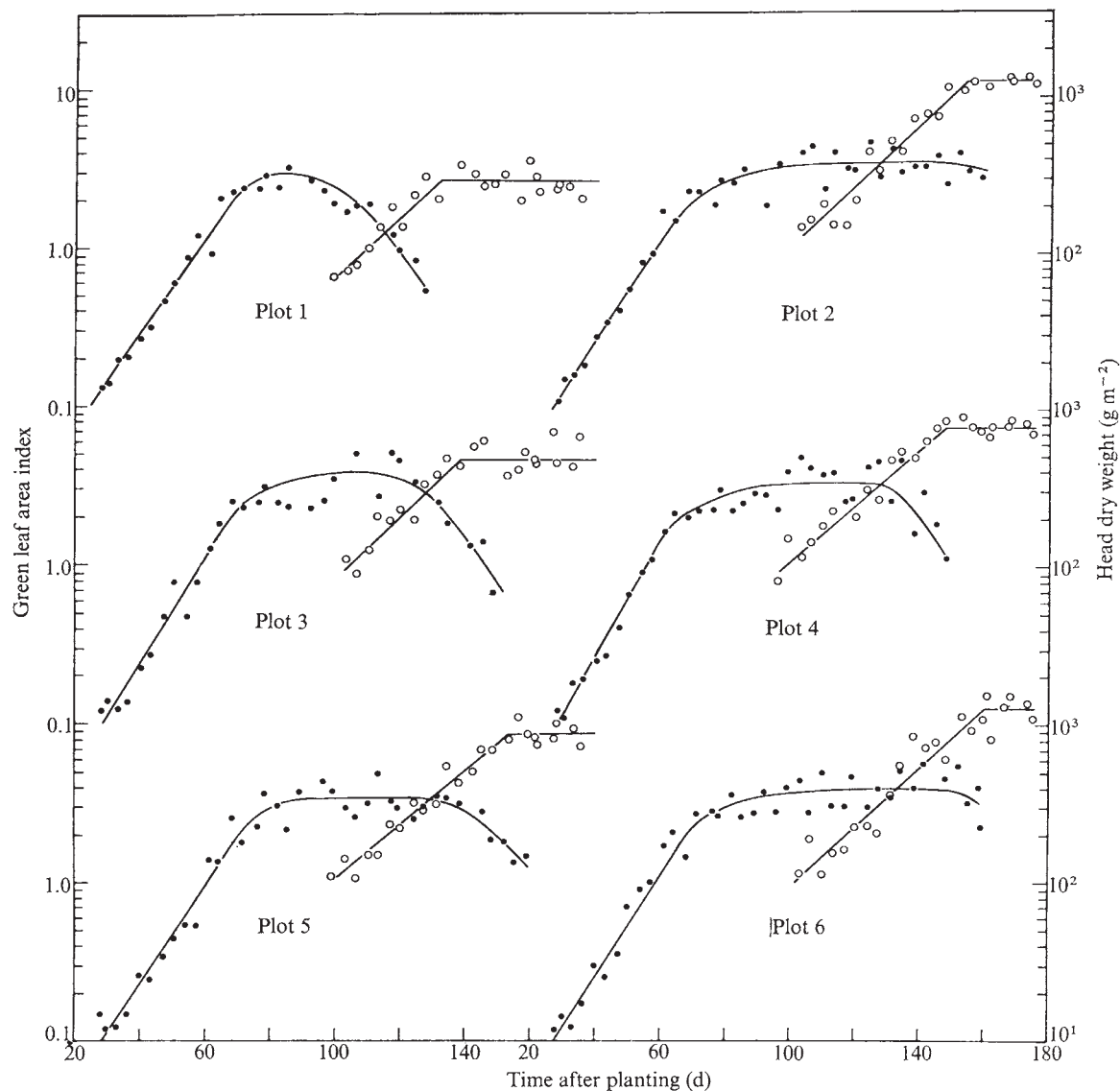


Fig. 2 Green leaf area index (●) and head dry weight (○) accumulation of six differentially-irrigated plots of Produra wheat grown at Phoenix, Arizona as a function of days after planting.

to standardise our data in an albedo format; but many other means of standardisation could be used which do not require ground-based measurements. Normalisation to some constant albedo feature such as a dry lake bed, a rock outcrop, a non-eutrophic lake, or even a man-made surface could be accomplished entirely by remote sensing. In such a programme, however, over-flights may need to be more frequent than the every-9-d coverage now provided by the Landsat satellite systems, in order accurately to establish albedo trends before and after head ripening to determine the critical minimum albedo value required to estimate final grain yield.

Our present approach is intended for use only with grain crops, but might be extended to other types of crops. For two reasons, our data suggest that the linear relationship shown in Fig. 4 may be applicable to durum wheat (Produra) grown anywhere in the world. First, the low albedo values after the early rains that reduced soil albedo were not apparent beyond day 80, when all plots had developed green leaf area indices of 2.5 or more. Thus, beyond this stage of growth, soil albedo seems to be of no consequence. Second, there were significant differences in the numbers of heads produced by each plot, yet no explicit accounting for this fact was required by us in correlating albedo with final grain yield. Thus, density of planting may also prove to be of little consequence.

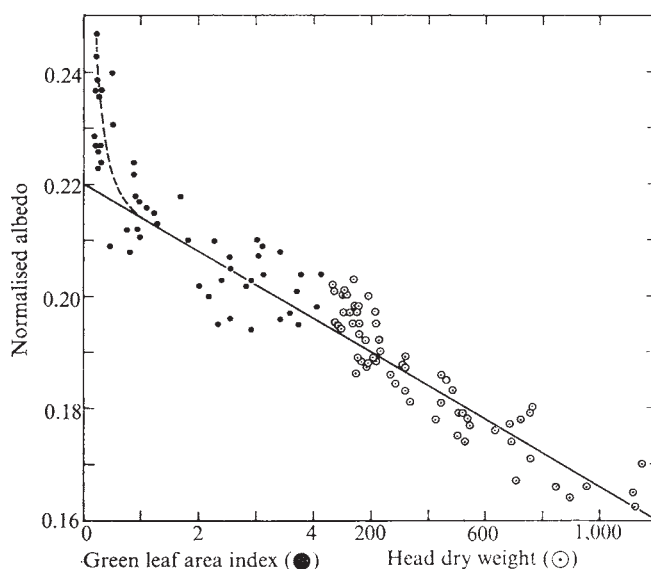


Fig. 3 Clear-day normalised albedo against green leaf area index and head dry weight of Produra wheat grown at Phoenix, Arizona.

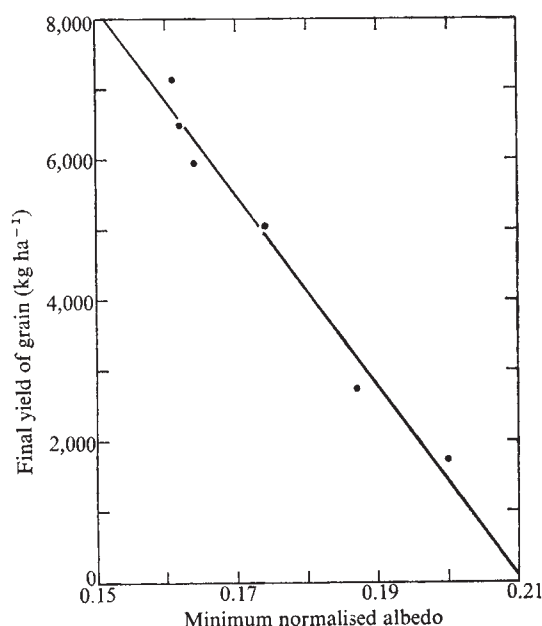


Fig. 4 Final grain yield of six differentially-irrigated plots of Produr wheat grown at Phoenix, Arizona against the minimum value of normalised albedo measured over each plot. $y = 28,370 - 134,700x$, $r_{y-x} = -0.986$.

Finally, we believe that measurement of reflected solar radiation is one viable means for the remote sensing of crop yields. Together with estimation techniques based on remote surface temperature measurement³, it should make possible a remote sensing system for the evaluation of agricultural productivity on a global scale.

S. B. IDSO
R. J. REGINATO
R. D. JACKSON

US Water Conservation Laboratory,
4331 East Broadway,
Phoenix, Arizona 85040

Received 11 October 1976; accepted 1 February 1977.

¹ Hammond, A. L. *Science* 188, 434 (1975).

² Idso, S. B., Jackson, R. D., Reginato, R. J., Kimball, B. A. & Nakayama, F. S. *J. appl. Meteorol.* 14, 109 (1975).

³ Idso, S. B., Jackson, R. D. & Reginato, R. J. *Science* (in the press).

Experimental transmission of *Leishmania chagasi*, causative agent of neotropical visceral leishmaniasis, by the sandfly *Lutzomyia longipalpis*

THE phlebotomine sandfly *Lutzomyia longipalpis* Lutz & Neiva (Diptera: Psychodidae) has long been suspected to be the vector of *Leishmania chagasi* Marques da Cunha & Chagas, in the principal foci of human visceral leishmaniasis ('kala azar') in Latin America¹. In general, the distribution of this sandfly coincides with the human disease, and the insect is a common domestic or peridomestic species which feeds avidly on man. *Lu. longipalpis* has been found naturally infected with promastigote flagellates which could have been those of *L. chagasi*²⁻³, but on none of these occasions was the parasite inoculated into susceptible laboratory animals and its true identity remains unknown. Previous attempts to experimentally transmit *L. chagasi* by the bite of *Lu. longipalpis* have failed⁴. We report here the first experimental transmissions of the parasite by laboratory-bred *Lu. longipalpis*, thus

providing further evidence that this sandfly is a major vector of American visceral leishmaniasis.

In transmission experiments we used an isolate (L.d./PP-75) of *L. chagasi* obtained from a child from Ituaçu, Bahia State, Brazil. The parasite was passed from the original culture medium in which it was isolated, directly into hamsters: it had been passaged in these animals on only three occasions before the present experiments were commenced. It is a particularly virulent organism in the hamster, the animals usually dying of a fulminating infection in 5-7 months after the intraperitoneal inoculation of spleen suspension from infected animals.

Our closed colony of *Lu. longipalpis* was established from flies collected in houses and farm buildings in Morada Nova, Ceara State, north-east Brazil: at the time of this work it was in its twenty-third generation⁵.

The infection rate obtained by feeding *Lu. longipalpis* on the skin of anaesthetised, infected hamsters was low (about 10%) and we felt that if successful transmission was to be achieved we needed much larger numbers of heavily infected sandflies than this method could provide. Following the findings of Adler and Theodor⁶, one of us (R.D.W.) tried feeding *Lu. longipalpis* on defibrinated rabbit blood through a variety of membranes. It was found that large numbers would readily engorge, and that the most suitable membrane was freshly removed chick skin. Temperature of the blood was maintained at 37 °C by use of an immersion thermostat apparatus (Thermomix II). We felt, therefore, that by using this system with a suspension of amastigotes of *L. chagasi* in rabbit blood we could probably ensure that all fed sandflies were infected.

Hamsters with 5-6-month-old infections usually showed spleens that were packed with parasites. The spleen of one such animal was triturated in a glass tissue-grinder with 4.0 ml of sterile, defibrinated blood which had been inactivated at 56 °C for 20 min (Fig. 1a). Approximately 300 *Lu. longipalpis* were fully engorged on this suspension, through a chick membrane, and subsequently maintained in individual tubes⁷.

Dissections of sandflies confirmed a 100% infection rate: by 5 d after the infective feed they were showing massive promastigote infections throughout the midgut and up to the anterior part of the cardia, where they appeared as stubby or rounded forms attached to the intestinal wall.

Each day, all those sandflies which had oviposited were released into a cage into which was placed a clean hamster, anaesthetised with Nembutal (Abbott). The animal was placed on its back and secured to a cork board with adhesive tape: its abdomen was shaved, to offer maximum surface area to the sandflies, and all re-feedings were carried out in the dark. Twelve transmission attempts were made, using batches of 4-40 infected flies. The first was on day 7 after the infective feed, and the rest on days 8-17 and 19.

Numerous flies were dissected during the experiment in order to follow the development of *L. chagasi*, and in particular to see when flagellates first gained entry to the pharynx and proboscis.

On day 5 of the infection the single fly examined showed enormous numbers of long thin promastigotes (Fig. 1b) in the midgut, with somewhat shorter, dividing forms in a more anterior position in the cardia (Fig. 1c). No parasites were seen in the pharynx, cibarium or proboscis. Two insects dissected on day 6 showed a similar pattern of development.

On day 7, one out of three *Lu. longipalpis* showed three promastigotes swimming freely in the pharynx. These parasites (Fig. 1d) were notably smaller than the form seen in the midgut of the same fly, and they moved with extraordinary rapidity. No parasites were seen in the proboscis of this insect, and the two other sandflies

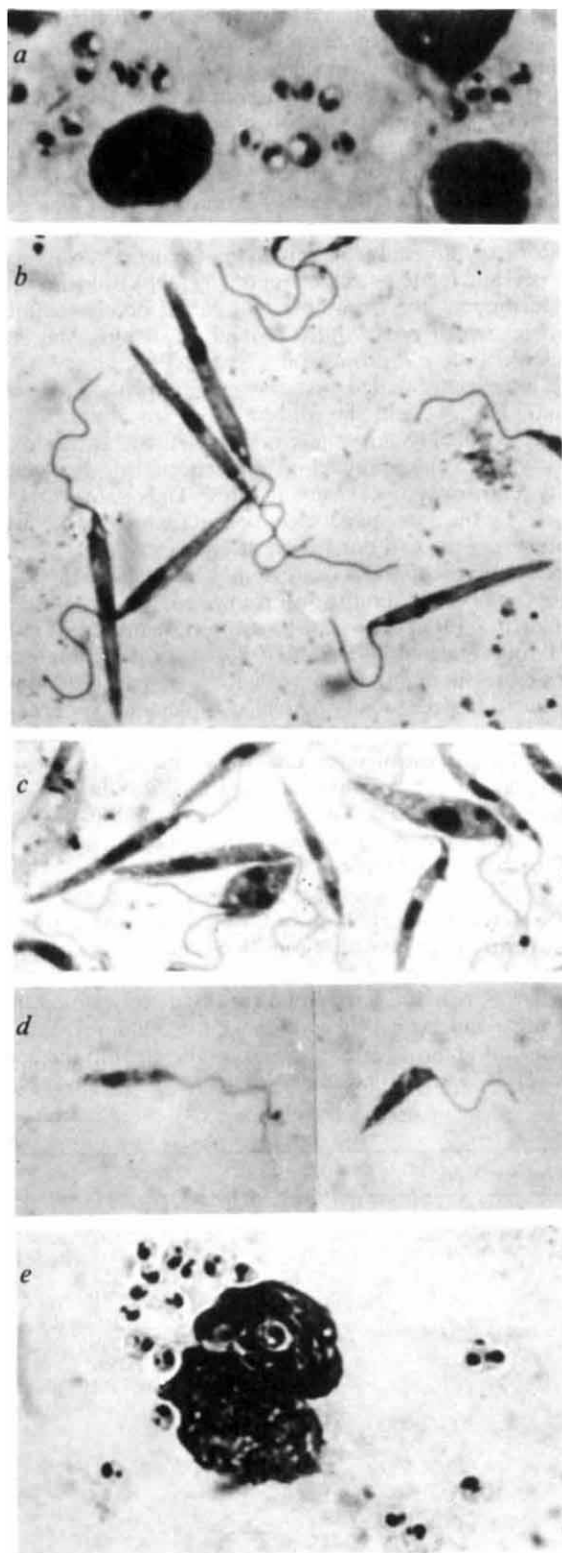


Fig. 1 Experimental transmission of *Leishmania chagasi* by the bite of the sandfly *Lutzomyia longipalpis*. *a*, Stained smear of a suspension of amastigotes of *L. chagasi* obtained by triturating the spleen of an infected hamster in inactivated rabbit blood. This material was used to infect the sandflies, through a chick-skin membrane. *b*, Long, thin promastigotes of *L. chagasi*, as seen in the midgut of *Lu. longipalpis*, 5 d after the infective feed. *c*, Shorter flagellates, some dividing, from the cardia of the same fly. *d*, Small promastigotes of *L. chagasi* from the pharynx of *Lu. longipalpis* on day 7 of infection. *e*, Impression smear of the spleen of a hamster, approximately 4 months after the animal was bitten by three infected *Lu. longipalpis*: intracellular and extracellular amastigotes of *L. chagasi*. All preparations fixed in absolute methyl alcohol and Giemsa-stained ($\times 1,420$).

dissected at the same time had flagellates no further forward than the cardia. On day 8, a single insect examined again showed a massive infection of the midgut, including the cardia, but absence of parasites in the foregut. Subsequently, pharyngeal forms were once more seen, in one fly, on day 10. Six others dissected during days 11–13 showed only midgut infections.

The first occasion on which flagellates were seen in the proboscis was on day 14, when two flies were dissected: both showed the usual, heavy midgut development of *L. chagasi* and, in addition, a number of shorter and more active forms in the pharynx. In one of the sandflies, however, a single parasite was seen moving rapidly up and down the length of the proboscis.

No foregut forms were seen in two insects examined on day 15, but light pharyngeal and proboscis infection was found in one sandfly on day 16. Finally, two flies dissected on day 17 showed parasites restricted to the midgut; no sandflies were examined beyond this date.

One hamster was found moribund, just over 4 months after three infected *Lu. longipalpis* had fed on it. Necropsy showed very large numbers of amastigotes of *L. chagasi* in impression smears of the spleen (Fig. 1*e*), and slightly fewer in the liver: culture of splenic tissues in blood-agar medium (NNN) produced growth of promastigotes. The remaining 11 hamsters were killed and examined at various dates from 5–6 months after the experiment, and four more proved to be infected: they showed variable numbers of amastigotes in the spleen, liver and bone marrow, and in all cases the spleen was the most heavily parasitised organ.

Thus at least 5 separate transmissions were achieved after feeding a total of 34 infected *Lu. longipalpis* on 12 hamsters. As up to six infected sandflies were on some occasions persuaded to feed on a single hamster, it is very likely that the number of transmissions was in fact more than five.

All transmissions were made during the first blood meal after the insects were infected, on days 7, 11, 13, 14 and 15. The transmission as early as 7 d after the infective meal is particularly interesting, for although scanty pharyngeal parasites were seen in one fly at this time no proboscis infections were recorded until the fourteenth day. Possibly the previous presence of flagellates in the proboscis is not prerequisite to transfer of the parasite, there simply being a surge of parasites forward from the pharynx, into the mouthparts, during the feeding process.

The incubation of the infection to a fatal stage in the hamster following transmission by the bite of the sandfly did not differ significantly from that obtained after the direct passage of amastigotes by syringe. This seems surprising, considering the very large number of amastigotes usually injected—with a triturate of hamster spleen, for example. It suggests that many more promastigotes may be inoculated by the sandfly vector than we have previously suspected.

We consider that the luxuriant growth of *L. chagasi* in *Lu. longipalpis*, the consistent migration of the parasite to the anterior station of the intestine and the biting mouthparts, the relative ease with which transmission was achieved by the bite of the insect, together with previous epidemiological evidence, confirm that *Lu. longipalpis* is a major vector of *L. chagasi* in endemic areas of human visceral leishmaniasis in Latin America. A detailed account of the morphological development of *L. chagasi* in *Lu. longipalpis* will be published elsewhere.

This work was supported by the Wellcome Trust and the Instituto Evandro Chagas of the Fundação Serviços de Saúde Pública (F. SESP) of Brazil. We thank Roberto Naiff, Maricleide Farias, Cristina Loureiro, Cesarina Arcanjo and José Almeida for technical assistance. Aluizio

Figueiredo was responsible for photography. Dr Robert Sogayar provided the *L. chagasi* isolate.

R. LAINSON
R. D. WARD
J. J. SHAW

Department of Parasitology,
Instituto Evandro Chagas, C.P. 3,
Belém 66.000, Pará, Brazil

Received 21 December 1976; accepted 9 February 1977.

- ¹ Deane, L. M., *Publ. Servico Nacional de Educacao Sanitaria, Rio de Janeiro, Brasil*, 1-162 (1956).
- ² Pifano, F. *Boln Ent. venez.* 2, 99-102 (1943).
- ³ Deane, M. P. & Deane, L. M. *O Hospital, Rio de Janeiro* 45, 697-701 (1954).
- ⁴ Sherlock, I. A. & Sherlock, V. A. *Rev. Soc. bras. Med. trop.* 6, 35-39 (1972).
- ⁵ Ward, R. D. thesis, London Univ. (1974).
- ⁶ Adler, S. & Theodor, O. *Ann. trop. Med. Hyg.* 21, 111-134 (1927).
- ⁷ Ward, R. D. *Mems Inst. Oswaldo Cruz* 70, 15-28 (1972).

Conjugal transfer of R plasmids in *Neisseria gonorrhoeae*

THE appearance of penicillin-resistant, β -lactamase-producing strains of *Neisseria gonorrhoeae* has been the cause of considerable concern¹. We have recently shown that β -lactamase production by these gonococci is plasmid mediated². Two distinct R plasmids have been characterised; one, with mass 4.4×10^6 was common in gonococci isolated from military personnel in the Far East and their sexual contacts. The other plasmid with mass 3.2×10^6 was found in a strain isolated from a woman in London³ and we have also found this plasmid species in clinical isolates from Liverpool⁴. DNA-DNA duplex studies have revealed that both gonococcal R plasmids possess a significant portion (about 40%) of the transposable sequence, TnA, which includes the β -lactamase gene commonly found on R plasmids of the Enterobacteriaceae, *Pseudomonas aeruginosa* and *Haemophilus influenzae*⁵.

While studying the β -lactamase producing gonococci from

the Far East we noted that they differed in nutritional requirements and in chromosomal antibiotic resistance genes, suggesting that there had been considerable dissemination of the R plasmid rather than simply the spread of a single ancestral clone. Investigations into the possible modes of dissemination led us to discover that a 24.5×10^6 molecular mass indigenous plasmid possessing sex factor activity seems to play a significant part in the transfer of R plasmids from gonococci to other strains and bacterial species.

Historically, the only known mode of genetic transfer among gonococci and related species has been by DNA transformation⁶. Transformation for chromosomal genes occurs readily to gonococci which possess pili (T1 and T2 strains) but not to strains which do not possess pili (T3 and T4 strains)⁶.

We, therefore, studied first the possibility that gonococcal plasmid DNA could be disseminated by transformation. Purified covalently-closed plasmid DNA was isolated from several β -lactamase-producing gonococci by dye buoyant density centrifugation of cleared lysates⁷. The plasmid DNA was analysed by the agarose gel electrophoresis method⁸ as well as by measuring the contour length of open circular DNA in the electron microscope⁹. As previously described by us and others¹⁰ gonococci almost invariably possess an indigenous plasmid 2.6×10^6 in mass. A 24.5×10^6 molecular mass indigenous plasmid is also found in about 5% of the penicillin-sensitive strains either alone or coexisting with the 2.6×10^6 plasmid⁹.

Thirteen independently isolated β -lactamase producing strains were examined. All strains contained the 2.6×10^6 plasmid. Each of the nine Far East strains carried the 4.4×10^6 R plasmid and the four strains isolated in Great Britain carried the 3.2×10^6 R plasmid. Three out of the nine Far East strains also contained the indigenous 24.5×10^6 plasmid while none of the Great Britain strains carried it.

Purified covalently-closed plasmid DNA and bulk cellular DNA isolated by the methods of Catlin¹¹ was used to transform the T1 form of the penicillin-sensitive (Pen^s), proline requiring (Pro⁻) *N. gonorrhoeae* strain F62. Using the transformation procedure of Sparling⁵, we could readily detect transformation for proline independence with the bulk cellular DNA (at a frequency of about 1%)⁵, but could not do so with the plasmid DNA. There was no detectable transformation to β -lactamase

Table 1 R plasmid transfer by *N. gonorrhoeae*

Donor strain†	Plasmid complement ($\times 10^6$)	Recipient strains	Plasmid complement ($\times 10^6$)	No. of penicillin-resistant cells per 10^8 recipient cells	Property of penicillin-resistant cells
<i>N. gonorrhoeae</i> CDC67 (T4, Pen ^r)	24.5	<i>N. gonorrhoeae</i> F62 (T4, pro ⁻ rif ^r nal ^r Pen ^s)	2.6	10,000*	Pen ^r Rif ^r Nal ^r Pro ⁻ ; 2.6×10^6 and 4.4×10^6 plasmid
Far East	4.4				
	2.6				
<i>N. gonorrhoeae</i> CDC67 (T4, Pen ^r)	24.5	<i>E. coli</i> HB101 (Amp ^s)	—	10	Pen ^r <i>E. coli</i> , 4.4×10^6 plasmid
Far East	4.4				
	2.6				
<i>N. gonorrhoeae</i> CDC36N (T4, Pen ^r nal ^r)	4.4	<i>N. gonorrhoeae</i> F62 (T4, pro ⁻ rif ^r nal ^r Pen ^s)	2.6	$< 10^{-8}$	—
Far East	2.6				
<i>N. gonorrhoeae</i> IPL (T4, Pen ^r arg ⁻ nal ^r)	3.2	<i>N. gonorrhoeae</i> F62 (T4, pro ⁻ rif ^r nal ^r Pen ^s)	2.6	$< 10^{-8}$	—
Great Britain	2.6				

Equal volumes of donor and recipient cells (1×10^7 colony forming units of each) were mixed in 10 ml of modified GC broth⁹ supplemented with 2×10^{-3} M CaCl₂ and 2×10^{-3} M MgCl₂. After overnight incubation the cells were collected by passing 3 ml of the culture through a membrane filter (Biorad 0.2 μ m). The filter was then placed on plates of double-strength Kellogg's medium⁹ and incubated for 6 h. The filters were removed and placed in 2 ml of GC broth and vigorously agitated to remove the cells. Samples of the mating mixture were plated on Kellogg's medium supplemented with 1μ g ml⁻¹ penicillin G and 1μ g ml⁻¹ nalidixic acid or 1μ g ml⁻¹ penicillin G and 1μ g ml⁻¹ rifampicin. Colonies appearing on the selective media were restreaked five times on the same selective media, and tested for β -lactamase activity using a chromogenic cephalosporin substrate³. The plasmid component of representative colonies showing β -lactamase activity was determined by Agarose gel electrophoresis.

Similar mating experiments were performed with *E. coli* HB101 as recipient and β -lactamase-producing recipients were selected by plating on MacConkey's agar containing 30μ g ml⁻¹ ampicillin. In some experiments, 50μ g ml⁻¹ of DNase (pancreatic DNase, Worthington) was added to the broth medium at the time of cell mixing, readjusted just before filtration, and added once again when the cells were removed from the filter.

*The data presented is from one set of experiments. In three later matings a range of 10^3 to 10^4 penicillin-resistant cells per 10^8 recipients were found in the CDC67-F62 matings.

†T4 colony type, see text: pro⁻, proline required; arg⁻, arginine required; Pen^r, penicillin resistant; Pen^s, penicillin sensitive; rif^r, rifampicin resistant; nal^r, nalidixic acid resistant; Amp^s, ampicillin sensitive.

production with either the cellular or plasmid DNA preparation. A number of other gonococcal recipients were then used, including a strain devoid of any plasmid species. None was transformed for β -lactamase production. In contrast, *E. coli* K-12 recipients were transformed to ampicillin resistance at a

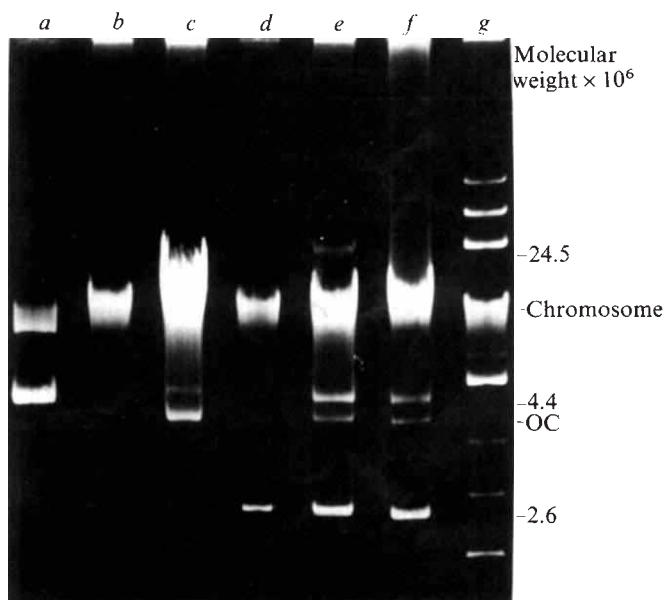


Fig. 1 Agarose gel electrophoresis of ethanol-precipitated DNA from cleared lysates of donor, recipient and penicillin-resistant recipient strains of *N. gonorrhoeae* and *E. coli*. *a*, Strain of *E. coli* HB101 penicillin-resistant recipient containing the 4.4×10^6 dalton plasmid derived from parental strain in slot *b*. Occasionally in the *E. coli* penicillin-resistant recipient lysates a plasmid band appears above the chromosome on the Agarose gel (not seen in this picture). The unusual band has been identified as an oligomer of the 4.4×10^6 plasmid by restriction enzyme analysis. *b*, Ampicillin-sensitive recipient strain of *E. coli* HB101 containing no plasmids. *c*, Ampicillin-resistant recipient containing the 4.4×10^6 dalton plasmid and the 2.6×10^6 cryptic plasmid, derived from parental strain in slot *d*. *d*, Penicillin-sensitive recipient strain of *N. gonorrhoeae* F62 containing the 2.6×10^6 cryptic plasmid. *e*, Penicillin-resistant donor strain *N. gonorrhoeae* CDC 67 containing the 24.5×10^6 , 2.6×10^6 indigenous plasmids and the 4.4×10^6 R plasmid. *f*, Penicillin-resistant donor strain of *N. gonorrhoeae* CDC36N containing the 2.6×10^6 indigenous plasmid and the 4.4×10^6 R plasmid. *g*, Standard plasmid DNAs; ranging in size from 62×10^6 (uppermost band) to 1.9×10^6 (lowest band). OC refers to the open circular form of the 2.6×10^6 cryptic plasmid.

frequency of about 10^3 cells per microgram of purified plasmid DNA, and were found to possess only a single R plasmid. We concluded, therefore, that the gonococci were refractory to transformation by closed circular plasmid DNA and the mechanism for the dissemination of the gonococcal R plasmids was not likely to be transformation.

The relatively high incidence of the 24.5×10^6 indigenous plasmid among the β -lactamase producing strains suggested the possibility that this plasmid might possess sex factor activity and that R plasmid transmission between gonococci might be mediated by conjugation. Donor and recipient strains exhibiting the non-piliated T4 colonial morphology were isolated. The donor strains were all β -lactamase producers and either required arginine (Arg⁻) or required no added amino acids to grow on Catlin's defined medium¹¹. The donor strains were sensitive to $1 \mu\text{g ml}^{-1}$ of nalidixic acid (Nal) and/or $1 \mu\text{g ml}^{-1}$ of rifampicin (Rif). The recipient strain used was the T4 form of the strain F62 (Pro⁻ Rif⁺ Nal⁺). Matings were performed by a variation of the filter method used by Van Klingeren *et al.* to examine the conjugative properties of *H. influenzae* R plasmids¹². Similar mating experiments were also performed using *E. coli* strain HB101 (ref. 7) as a recipient.

The results of several representative mating experiments are summarised in Table 1. Plasmid complements of donor and recipient cells as well as resistant cells recovered from mating

mixtures are illustrated in Fig. 1. β -lactamase-producing *N. gonorrhoeae* and *E. coli* recipient cells were recovered in the matings. However, successful transfer of the R plasmid was observed only when the donor gonococcal strain possessed the 24.5×10^6 plasmid as well as the R plasmid. No β -lactamase-producing recipients could therefore be isolated from matings in which isolates from Great Britain were used as donors nor with donor strains of Far East origin which lacked the 24.5×10^6 plasmid. As shown in Fig. 1, the acquisition of β -lactamase biosynthesis by recipients was associated with the appearance of R plasmid DNA. The selection of β -lactamase-producing gonococci or *E. coli* recipients was not influenced by the presence of DNase (data not shown).

The data suggests that the indigenous 24.5×10^6 plasmid plays a significant part in the transfer of R plasmids from gonococci to other strains and species. We hypothesise that this large cryptic plasmid possesses sex factor activity and is able to mobilise or otherwise promote the transfer of the smaller coexisting R plasmid by conjugation. It seems improbable that transformation is involved in the transfer of R plasmids in mixed culture because of the failure to demonstrate the phenomenon with purified plasmid DNA, because the T4 donor and recipient cells are not normally competent for DNA transformation⁶, and because plasmid transfer in mixed cultures was uninfluenced by DNase. Phage-mediated transduction could be an alternative explanation for our results, although to our knowledge no gonococcal phage has been described and any transducing phage would have to have the unusual ability to infect both *E. coli* and *Neisseria*. Moreover, cell-free filtrates of the donor strains could not transfer the R plasmid to *N. gonorrhoeae* or *E. coli* recipient cells (data not shown).

It thus seems likely that conjugation is the mechanism of R plasmid transfer in the gonococci and is promoted by the 24.5×10^6 plasmid. In overnight matings the 24.5×10^6 plasmid and 2.6×10^6 plasmid are often also detected in *Neisseria* but not *E. coli* which received the R plasmid. Recent data has shown that the 24.5×10^6 plasmid is capable also of promoting the transfer of chromosomal genes. The finding that R plasmids of *N. gonorrhoeae* seem to be readily transmitted among various strains of gonococci has serious epidemiological implications. Not only must we be concerned about the possibility of widespread dissemination of resistance but also the extension of resistance to related microorganisms such as *N. meningitidis* which are currently penicillin sensitive. We feel this change is required in light of new data which has shown that in overnight mating significant numbers of gonococcal recipients received the 24.5 MDal plasmid and thus are capable of transmitting the R plasmid to new strains. This has very serious epidemiological consequences.

We thank Dr C. Thornsberry, Center for Disease Control, Atlanta, Georgia for providing us with the penicillin-resistant strains of *N. gonorrhoeae*, and Dr Lynn Elwell and Dr Leonard Mayer for help and suggestions. This work was supported by a grant from the National Institutes of Allergy and Infectious Diseases.

MARILYN ROBERTS
STANLEY FALKOW

Department of Microbiology and Immunology,
University of Washington School of Medicine,
Seattle, Washington 98195

Received 21 January; accepted 3 February 1977.

- Ashford, W. A., Golash, R. C. & Hemming, V. G. *Lancet* ii, 657-658 (1976).
- Elwell, L. P., Roberts, M., Mayer, L. W. & Falkow, S. *Antimicrob. Agents Chemother.* (in the press).
- Phillips, I. *Lancet* ii, 656-657 (1976).
- Percival, A. *et al.* ii, 1379-1382 (1976).
- Sparling, P. F. *J. Bact.* 92, 1364-1371 (1966).
- Kellogg, D. S., Jr, Peacock, W. L., Deacon, W. E., Brown, L. & Pringle, C. I. *J. Bact.* 85, 1274-1279 (1963).
- DeGraaff, J., Elwell, L. P. & Falkow, S. *J. Bact.* 126, 439-446 (1976).
- Meyers, J., Sanchez, D., Elwell, L. P. & Falkow, S. *J. Bact.* 127, 1529-1537 (1976).
- Mayer, L. W., Holmes, K. K. & Falkow, S. *Infect. Immun.* 10, 712-717 (1973).
- Engelkirk, P. G. & Schoenhard, D. E. *J. infect. Dis.* 127, 197-200 (1973).
- Catlin, B. W. *J. Infect. Dis.* 128, 178-194 (1973).
- Van Klingeren, B., Van Embden, J. D. A. & Dessens-Kroon, M. *Antimicrob. Agents Chemother.* (in the press).
- Kammer, B. T., Preston, D. A., Turner, J. R. & Hawley, L. C. *Antimicrob. Agents Chemother.* 8, 91-94 (1975).

Thymus metabolises progesterone—possible enzymatic marker for T lymphocytes

FEMALES of the athymic mutant strain of nude mice show severe deficiencies in reproductive function. The ovaries and the uterus are abnormally small¹, puberty is delayed², oestrous cycles are abnormal and mature females are usually sterile^{1,3}. These abnormalities can be prevented by grafting thymic tissue at birth^{2,3}. Similar abnormalities result from neonatal thymectomy of normal female mice, and can be corrected by thymus re-grafting up to 7 d age^{2,4}. These observations suggest an involvement of the thymus in gonadal development and function. Conversely, certain sex hormones are known to cause atrophy of the thymus⁵. Hitherto, however, there has been no evidence implicating the thymus of the infantile mouse in sex hormone metabolism. We report here the presence of 20 α -hydroxysteroid dehydrogenase (20 α -SDH) activity in the mouse thymus, an enzyme engaged in progesterone metabolism.

Thymus glands of 10-d-old C57BL female mice were homogenised in 0.01 M sodium phosphate, pH 7.4, 0.25 M sucrose and 0.001 M MgCl₂ and incubated at 37 °C with 1,2,6,7-³H-progesterone 1 $\times$ 10⁵ d.p.m. (specific activity 100 Ci mmol⁻¹, New England Nuclear) and varying amounts of unlabelled progesterone in the presence of 1 mM NADPH and 10 mM glucose-6-phosphate. After 5–60 min of incubation the resulting steroids were extracted with diethyl ether and separated by thin-layer chromatography in the system ether–chloroform (3:10), where the substrate is separated from reduced metabolites of progesterone. Radioactive zones were located on the plates by scanning with a gas-flow counter (Packard model 7201) and the radioactive compounds were scraped off the plates, eluted with methanol and counted in a Packard TriCarb Scintillation counter.

Radiolabelled progesterone was converted to a radioactive product with chromatographic mobility (R_f = 0.33) identical to that of 20 α -hydroxypregn-4-en-3-one (20 α -OHP) and distinct from the residual substrate (R_f = 0.66). The compound did not dissociate from carrier 20 α -OHP when cochromatographed in two additional systems, benzene–acetone (4:1; R_f = 0.4) and cyclohexane–ethyl acetate (1:1; R_f = 0.46). A mixture of the TLC-purified radioactive product (92,000 d.p.m.) and authentic 20 α -OHP (31.5 mg; Ikapharm) was recrystallised thrice from aqueous methanol, yielding crystals of constant specific activity (2610 $\pm$ 130 d.p.m. mg; mean $\pm$ s.e.m.), and the specific activity of the second and third crops of crystals did not differ significantly from the corresponding mother liquors. These results indicate that the progesterone metabolite formed by the thymus tissue was 20 α -OHP.

The thymic enzyme catalyses the reaction in both directions: incubation of thymus homogenate with 20 α -OHP in the presence of NADP resulted in the production of progesterone (data not shown). When 20 α -OHP was incubated with thymus homogenate for 2 h in the presence of NADPH, no additional metabolites were detected.

Activity of thymic 20 α -SDH was linear up to 30-min incubation at 37 °C (Fig. 1a), and was directly proportional to protein concentration (measured by the method of Lowry *et al.*⁶), up to 600 μ g ml⁻¹ (Fig. 1b). The apparent K_m of the thymic 20 α -SDH was 21.6 $\pm$ 3.3 μ M (Fig. 1c), which is close to the value reported for the ovarian 20 α -SDH (ref. 7). The V_{max} of the thymic enzyme for the reductive step was 10.0 $\pm$ 0.4 nmol 20 α -OHP per mg protein per 20 min.

A comparison of 20 α -SDH activity in various organs of immature mice (Table 1) shows that enzyme activity of the thymocytes is at least 16-fold higher than in lung or kidney tissue. The thymocytes seem to be the source of the thymic enzyme, as their specific activity is approximately twice that of the whole gland. Considerable enzyme activity is present in the

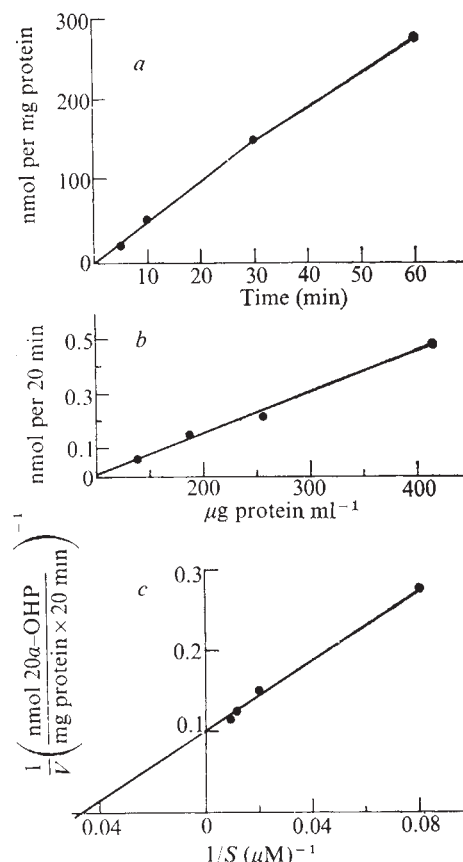


Fig. 1 Kinetic properties of thymic 20 α -hydroxysteroid dehydrogenase. The enzyme in homogenates of thymus glands from 10-d-old mice was assayed as described in the text, except that in *a* and *b* cold progesterone, 25 μ M, was used as substrate. *a*, Time course of enzyme activity (470 μ g protein per ml). Each point represents the mean of duplicate determinations. *b*, Effect of enzyme concentration on the rate of conversion of progesterone to 20 α -dihydroprogesterone. *c*, Double reciprocal plot of results according to the Lineweaver–Burk method. The apparent K_m was estimated according to Wilkinson¹⁴. The K_m was 21.6 $\pm$ 3.3 and V_{max} 10.0 $\pm$ 0.4 nmol 20 α -OHP per mg protein per 20 min.

spleen of normal C57BL mice, but is almost absent in the spleen of athymic (nude) mice (Table 1). These results suggest that 20 α -SDH is associated with thymus-processed T lymphocytes, and not with B lymphocytes. To clarify this point, the ability of thymocytes and spleen cells from phenotypically normal heterozygous (nu/+ C57BL) to reduce progesterone was compared to that of spleen cells from athymic (nu/nu) mice of the same age (6-week-old). The red blood cells were selectively lysed by treatment with 0.83 NH₄Cl and the remaining white cells were washed with phosphate-buffered saline, collected by centrifugation at 500g (10 min) and resuspended in RPMI1640 containing 1.0 μ M of labelled progesterone (400,000 d.p.m.).

Table 1 Distribution of 20 α -hydroxysteroid dehydrogenase

Organ	Specific activity (nmol 20 α -OHP produced per mg protein per h)
Thymus	4.39
Thymocytes	9.87
Spleen	0.25
Spleen of athymic mouse*	0.03
Lung	0.61
Kidney	0.29

Thymus and other organs from 10-d-old female C57BL (nu/+) mice were assayed for 20 α -SDH activity as described in Fig. 1 legend. The homogenates were incubated for 60 min.

* Athymic nude C57BL (nu/nu) mouse and the phenotypically normal heterozygous C57BL (nu/+) were obtained from G. L. Bomholtgaard, Denmark.

The rate of 20 α -OHP accumulation (pmol h⁻¹ per 5 $\times$ 10⁶ cells) during a 1-h incubation at 37 °C determined as described before, was 335.0 for thymocytes, 105.0 for spleen cells from nu/+ mice and < 5.0 for spleen cells from athymic nu/nu mice. The results indicate that in lymphocytes the enzyme is only represented in the T-cell type.

It has been reported that thymic tissue and lymphocytes are capable of reversibly oxidising cortisol to cortisone⁸, and that the activity of the enzyme that catalyses this oxidoreduction (11 β -hydroxysteroid dehydrogenase) was 0.128 nmol per 100 mg tissue per 3 h incubation, which is < 1/1000 of the activity of 20 α -SDH reported here. 20 α -SDH occurs in ovaries of many species and particularly in regressing corpora lutea of rodents, where its appearance signals the onset of luteolysis^{9,10}. To our knowledge, no other enzyme activity connected with sex steroid metabolism has been reported to operate in the thymus.

During the incubation of radiolabelled progesterone with thymocytes we were able to detect a small amount of 5 α -reductase activity, an enzyme which reduces progesterone to its 5 α -pregnane derivative. The major pregnane product formed was identified as 5 α -pregnane-3,20-dione by chromatography in 3 TLC systems, and by gas-chromatographic analysis of its bis-thioketal derivative¹¹. In addition, the partially purified product was recrystallised with authentic 5 α -pregnane-3,20-dione (Ikapharm) to constant specific activity.

The significance of the two progesterone metabolising enzyme activities in the thymus is not clear. Progesterone has been reported to be immunosuppressive^{12,13} at high concentrations, which are achieved, probably, only near its site of formation in the foeto-placental unit. It is therefore conceivable that an enzyme engaged in reducing progesterone may have a role in the defence mechanisms mediated by thymocytes. Experiments are in progress to relate the distribution of 20 α -SDH to that of known T-cell markers, such as θ antigen and responsiveness to plant lectins.

Y. WEINSTEIN
H. R. LINDNER

Department of Hormone Research,
The Weizmann Institute of Science,
Rehovot Israel

B. ECKSTEIN

Department of Zoology,
The Hebrew University of Jerusalem,
Jerusalem, Israel

Received 17 December 1976; accepted 18 February 1977.

- ¹ Shire, J. G. M. & Pantelouris, E. M. *Comp. Biochem. Physiol.* **47A**, 93–100 (1974).
- ² Besedowsky, H. O. & Sorkin, E. *Nature* **249**, 356–358 (1974).
- ³ Flanagan, S. P. *Genet. Res.* **8**, 295–309 (1966).
- ⁴ Nishizuka, Y. & Sakakura, T. *Science* **166**, 753–755 (1969).
- ⁵ Frey-Wettstein, M. & Craddock, C. G. *Blood* **35**, 257–271 (1970).
- ⁶ Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193**, 265–275 (1951).
- ⁷ Wilcox, R. B. & Wiest, W. G. *Steroids* **7**, 395–413 (1966).
- ⁸ Dougherty, T. F., Berliner, M. C. & Berliner, D. C. *Ann. N.Y. Acad. Sci.* **14**, 78–82 (1960).
- ⁹ Wiest, W. G. & Kidwell, W. R. in *The Gonads* (ed. McKern, K. W.) 295–325 (Appleton Century-Crofts, New York, 1969).
- ¹⁰ Lindner, H. R. & Lamprecht, S. A. *Adv. Biosci.* **4**, 419–428 (1969).
- ¹¹ Zmigrod, A. & Lindner, H. R. *Steroids* **8**, 119–132 (1966).
- ¹² Monroe, J. S. *J. reticuloendothel. Soc.* **9**, 361–375 (1971).
- ¹³ Carter, J. J. *Reprod. Fert.* **46**, 211–216 (1976).
- ¹⁴ Wilkinson, G. N. *Biochem. J.* **80**, 324–332 (1961).

Evidence for control of complement receptor rosette-forming cells by α - and β -adrenergic agents

PHARMACOLOGICAL agents such as dibutyryl cyclic AMP^{1,2}, cholera toxin^{3,4} and β -adrenergic stimulants⁵ have an effect on antibody production which can be inhibitory or enhancing, depending on the dose used and the time of addition of the agent. Such stimulants are known to inhibit the human T-lymphocyte receptor for sheep erythrocytes^{6,7}

and the murine lymphoma Fc receptor⁸, and it has been shown that the complement receptor on B lymphocytes has an important role in the cellular events leading to antibody production^{9,10}, thus emphasising the need to clarify its nature. We report here that the number of complement receptor rosette-forming cells (EAC-RFC) in suspensions of mouse spleen cells can be decreased by α -adrenergic stimulants and increased by β -adrenergic stimulants, respectively.

Male CBA mice, of 3–5 months, were killed by ether inhalation. The spleens were teased in Eagle's minimal essential medium (MEM) to give a single cell suspension. The cells were washed twice and the concentration adjusted to 5 $\times$ 10⁶ ml⁻¹ in Eagle's MEM containing 10% heat-inactivated foetal calf serum incorporating various concentrations of the pharmacological agents under study. After 3-h incubation at 37 °C in a humid atmosphere containing 5% CO₂ in air, the cells were washed three times and 5 $\times$ 10⁶ cells finally resuspended in 0.5 ml Eagle's MEM. The cell viability was judged to be 90–93% by the eosin dye exclusion test. For the estimation of complement rosettes, 0.5 ml aliquots of a 1% suspension of sheep red blood cells which had been freshly coated with rabbit anti-sheep red blood cell haemolysin (1:1,000) and fresh mouse serum (1:20) as a source of complement, were centrifuged with an equal volume of the lymphocyte suspension at 200g for 2 min. The cell pellets were incubated together at 37 °C for 15 min, resuspended, and the number of rosette-forming cells counted. A rosette-forming cell was defined as a white cell with three or more adherent erythrocytes. Each study was done in duplicate and 400 cells were counted per tube. To identify the type of cells involved in the rosettes, 10 mM EDTA was added at the time of the rosette assays¹¹. No significant change was observed. The rosette-forming cells in cultures of spleen cell suspensions incubated for 3 h with isoproterenol, adrenaline, noradrenaline, propranolol, dibutyryl cyclic AMP, dibutyryl cyclic GMP, aminophylline (all Sigma) or phentolamine (Regitin, CTBA) were therefore thought to be lymphocytes.

The effect on EAC-RFC of the β -adrenergic stimulator, isoproterenol, is shown in Fig. 1a. The number of EAC-RFC rose to a peak in a concentration of 5 $\times$ 10⁻⁵ M isoproterenol, and declined to almost to control values at 10⁻¹⁴ M. Isoproterenol is known to increase the concentration of intracellular cyclic AMP by stimulating adenylyl cyclase activity¹². The addition of dibutyryl cyclic AMP, however, had no effect on the number of EAC-RFC (Fig. 1h). When 10⁻⁴ M aminophylline, a phosphodiesterase inhibitor¹³, was added with dibutyryl cyclic AMP, the number of EAC-RFC was unchanged (Table 1). The α -stimulant noradrenaline inhibited slightly the number of EAC-RFC. Adrenaline, which is thought to stimulate both the α - and β -adrenergic receptors, had no effect on the number of EAC-RFC (Fig. 1e). Presumably the effect of stimulating both receptors at the same time is to cancel out any effect on the number of EAC-RFC. It can be demonstrated, however, that adrenaline in such a situation is active, since its addition simultaneously with 10⁻⁵ M phentolamine (an α -blocker)¹⁴ or 10⁻⁵ M propranolol (a β -blocker)¹⁵ results in a significant increase or decrease, respectively, in the number of EAC-RFC (Fig. 1f,g). The concentrations of propranolol and phentolamine were such that neither drug on its own had a significant effect on EAC-RFC. It is probable that normal foetal calf serum contains a small quantity of adrenalin. Therefore the following experiment was done to clarify the effect of α -stimulant (noradrenaline) or β -stimulant (isoproterenol) in the foetal calf serum culture medium. When 10⁻⁵ M phentolamine was added to the medium containing isoproterenol, EAC-RFC increased in number as far as the concentration of the latter was

Table 1 Effect of pharmacological agents on complement rosette formation of mouse spleen cell suspensions at 37 °C for 3 h

	Mean % rosettes ±s.d.		Mean % rosettes ±s.d.
5×10^{-5} M isoproterenol (6)	$33.80 \pm 2.96^\dagger$	Control (C) (6)	22.74 ± 1.81
10^{-15} M isoproterenol			
+ 10^{-5} M phentolamine (3)	$32.91 \pm 1.80^\ddagger$	C (3)	21.37 ± 1.83
5×10^{-5} M noradrenaline (7)	$15.35 \pm 1.90^\ddagger$	C (7)	23.22 ± 1.70
10^{-6} M noradrenaline			
+ 10^{-5} M propranolol (3)	$8.16 \pm 1.80^\ddagger$	C (3)	21.08 ± 0.52
10^{-6} M adrenaline (5)	23.10 ± 0.98	C (5)	24.1 ± 0.56
10^{-6} M adrenaline			
+ 10^{-5} M propranolol (4)	$11.31 \pm 2.30^\ddagger$	C (4)	20.75 ± 1.85
10^{-5} M adrenaline			
+ 10^{-5} M phentolamine (5)	$30.10 \pm 3.02^\ddagger$	C (5)	21.05 ± 0.73
10^{-8} M dibutyl C-AMP (6)	21.30 ± 2.00	C (6)	20.29 ± 1.33
+ 10^{-5} M aminophylline (6)	20.54 ± 0.44	C (6)	20.29 ± 1.33

No. of duplicate cultures in brackets.

*Mean percentage rosettes = mean (no. of rosettes/no. of spleen cells) $\times$ 100.

$^\dagger P < 0.001$.

$^\ddagger P < 0.005$.

Statistical treatments were done with the difference between the mean value of rosette-forming cells of the test group and that of the control group.

kept between 10^{-11} M and 10^{-15} M (Fig. 1b). When 10^{-5} M propranolol was added to the medium in the presence of 10^{-4} M to 10^{-10} M noradrenaline, EAC-RFC decreased in number (Fig. 1d). Dibutyl cyclic GMP seemed to have no effect on EAC-RFC (Fig. 1i).

Our results show that the number of EAC-RFC in a suspension of mouse spleen cells can be increased by the addition of a drug which stimulates the β -adrenergic receptor. Conversely, an agent which stimulates the α -receptor depresses the number of EAC-RFC. Further confirmation of these findings has been obtained from results which have shown that adrenaline (a stimulator of

both α - and β -receptors) has no effect on the number of EAC-RFC in its own, but, with an α -blocker, adrenaline mimics a β -stimulator; when adrenaline and a β -blocker were used, the effect on the number of EAC-RFC is the same as that of an α -receptor stimulator (Fig. 1f and g).

α - and β -adrenergic receptors are thought to be regulatory (or receptor) units of the adenylyl cyclase¹⁶. Activation of the β -receptor stimulates the enzyme and causes an increase in the level of intracellular cyclic AMP, whilst stimulation of the α -receptor causes a decrease in intracellular cyclic AMP.

We were, however, unable to demonstrate an effect on

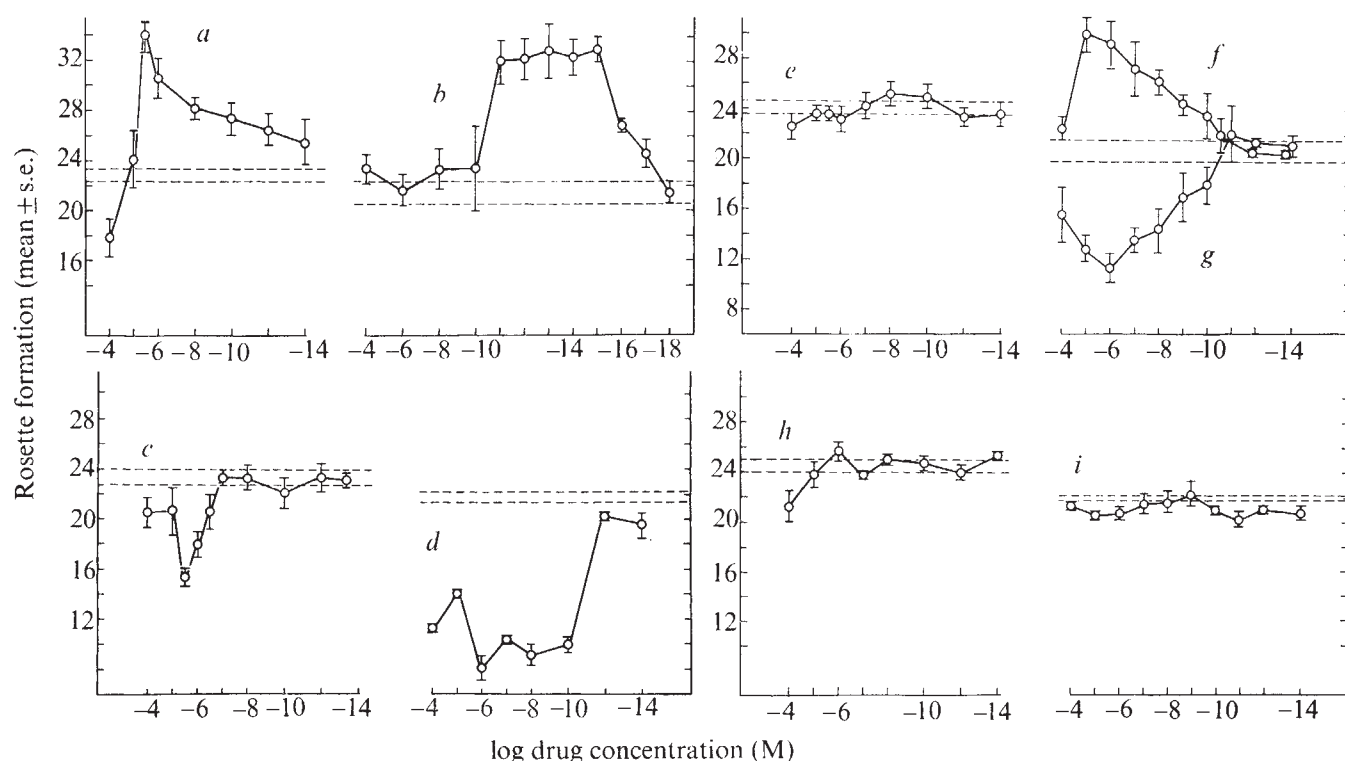


Fig. 1 Various concentrations of *a*, isoproterenol, *b*, isoproterenol + 10^{-5} M phentolamine, *c*, noradrenaline, *d*, noradrenaline + 10^{-5} M propranolol, *e*, adrenaline, *f*, adrenaline + 10^{-5} M phentolamine, *g*, adrenaline + 10^{-5} M propranolol, *h*, dibutyl cyclic AMP, *i*, dibutyl cyclic GMP were incubated with the mouse spleen cells at 5×10^6 cells per ml. Eagle's MEM (10% heat-inactivated foetal calf serum) in a 37 °C incubator in a 5% atmosphere for 3 h. After three washes EAC-RFC assay was performed as described in the text. Each point represents the mean rosettes, with $\pm$ s.e., of three to six animals. — = —, $\pm$ s.e. of control of each group.

complement receptor rosettes of dibutyl cyclic AMP, an agent believed to act beyond the level of adenyl cyclase. Furthermore, there was no effect when dibutyl cyclic AMP was added with aminophylline, an inhibitor of phosphodiesterase, which suppresses the breakdown of intracellular cyclic AMP and thus effectively increases its intracellular concentration¹³. The rosette-forming cells, therefore, seem to be affected, not by the increase in the intracellular cyclic AMP levels, but by the increase in adenyl cyclase activity itself. It is known that B lymphocytes with complement receptor can produce antibody to T-dependent antigens^{9,10}. On the other hand, catecholamines (adrenaline and noradrenaline) enter the blood stream, circulate throughout the body and affect various organs as hormones. We therefore suggest that in the immune system catecholamines influence the activity of complement receptor of B-lymphocyte, resulting in control of antibody formation.

This work was supported by a grant from the Ministry of Education of Japan. We thank Drs J. P. Durham and M. McLoughlin for discussion and Mrs H. Collin for technical assistance.

MASAHIKO ITO*
FREDERICK SLESS
DELPHINE M. V. PARROTT

University of Glasgow,
Department of Bacteriology and Immunology,
Western Infirmary,
Glasgow, UK

Received 15 January 1976; accepted 4 February 1977.

*Present address: Fukushima Medical College, Fukushima, Sugizuma-cho 5-75, Japan.

¹ Watson, J., Epstein, R. & Cohn, M. *Nature* **246**, 405-409 (1973).

² Teh, H. S. & Paetkau, V. *Nature* **250**, 505-507 (1974).

³ Cook, R. G., Stavitsky, A. B. & Schoenberg, M. D. *J. Immun.* **114**, 426-434 (1975).

⁴ Kately, J. R., Kasarov, L. & Friedman, H. J. *Immun.* **114**, 81-86 (1975).

⁵ Braun, W. *Ann. N.Y. Sci.* **207**, 17-28 (1973).

⁶ Chisari, F. V. & Edgington, T. S. *J. exp. Med.* **140**, 1122-1126 (1974).

⁷ Galant, S. P. & Remo, R. A. *J. Immun.* **114**, 512-513 (1975).

⁸ Zuckerman, S. H. & Douglas, S. D. *Nature* **255**, 410-412 (1975).

⁹ Dukor, P., Dietrich, F. M., Gissler, R. H., Schuman, G. & Bitter-Suermann, D. *Prog. Immun.* **113**, 99-109 (1974).

¹⁰ Arnaiz-Villena, A., Playfair, J. H. L. & Roitt, I. M. *Clin. exp. Immun.* **20**, 375-378 (1975).

¹¹ Bianco, C., Patrick, R. & Nussenzweig, V. *J. exp. Med.* **132**, 702-720 (1970).

¹² Butcher, R. W. & Sutherland, E. W. *Ann. N.Y. Acad. Sci.* **139**, 849-859 (1967).

¹³ Butcher, R. W. & Sutherland, E. W. *J. biol. Chem.* **237**, 1244-1250 (1962).

¹⁴ Roberts, G., Richardson, A. W. & Green, H. D. *J. Pharmac. exp. Therap.* **105**, 466-476 (1952).

¹⁵ Black, J. W., Crowther, A. F., Shanks, R. G., Smith, L. H. & Dornhorst, A. C. *Lancet* **i**, 1080-1081 (1964).

¹⁶ Robinson, G. A., Butcher, R. W. & Sutherland, E. W. *Ann. N.Y. Acad. Sci.* **139**, 703-723 (1967).

report here that systemic injections of α -MSH in female rats affect the arcuate DA neurones as well as those of substantia nigra. The latter were studied in order to check for eventual parallel responses in an extrahypothalamic DA system. The peptide effect seems to depend upon the integrity of projections ascending from the lower brainstem. Cholinergic systems seem to participate in its manifestation.

We studied female SIV (Sprague-Dawley-Ivanovas) rats kept in groups of littermates under controlled illumination and temperature conditions (lights on 06.00-20.00; $22 \pm 1^\circ\text{C}$). The animals were ovariectomised at the age of 4-5 months, 3 weeks before the experiments, and pretreated for one day with oestradiol-dipropionate (5 μg s.c.) and progesterone (2 mg s.c.), since we had used the same animal model in our studies on extrahypothalamic influences. The functional state of the DA neurone groups was assessed by a microfluorimetric procedure based on the histochemical fluorescence method of Falck and Hillarp. The distributions of fluorescence intensity of individual neurones are characteristic for a given functional state and neurone group. Studies on nigral DA neurones have revealed a close relation between mean intensity and mean firing rate; in agreement with earlier observations, a rise in mean intensity accompanied an increase in mean firing rate⁷.

In the higher dose range which corresponds to that used in most behavioural experiments, α -MSH injected intraperitoneally (i.p.) (between 15.00 and 17.00) induced a marked rise in the mean fluorescence intensity of both DA neurone groups within 30 min (Fig. 1a, $P < 0.01$ for 100 μg per kg body weight; similar effect for 33 μg per kg but significant only in the arcuate nucleus). At lower doses, only the arcuate DA neurones responded ($P < 0.01$). This difference may have resulted from different sensitivities of the projection systems involved. The observation of a response of nigral DA neurones 15 min after injection of a lower dose in one rat suggests that the nigral reaction might also be more transient. If the relation established between cellular fluorescence intensity and neuronal activity⁷ holds true also in the present case, the reaction of the arcuate DA neurones indicates an activation. Since DA inhibits MSH release⁴, this corresponds to the activation of a negative feedback mechanism. The similar response of the nigral DA neurones may be linked with certain extrahypothalamic effects of the peptide. In analogy to the situation in the arcuate DA neurones, one may ask whether the nigral reaction might likewise reflect the activation of a compensatory mechanism. An aggravation of tremor by MSH has been noted in Parkinson patients⁸. Our findings are at variance with observations on repeated injections of α -MSH in male rats, where DA turnover in midbrain and striatum remained unchanged⁹. Whether this discrepancy is due to differences in the experimental arrangement or in the methods used for analysis of DA systems, cannot yet be decided. It seems conceivable that the hormonal state of the animal might influence peptide effects either qualitatively or quantitatively.

The response of the arcuate DA neurones cannot be expected to be specific for α -MSH, since this neurone group seems to be involved in the control of various pituitary hormones. Prolactin (secretion of which is likewise inhibited by this DA system) showed a similar effect (Fig. 1b, $P < 0.01$ compared with solvent- and to saline-injected groups). It can also be regarded as the activation of a negative feedback mechanism¹⁰. More surprising is the response of the nigral DA neurones ($P < 0.01$ versus solvent group, $P < 0.05$ versus saline group). It may reflect a direct effect of prolactin or be due to some more complex neuroendocrine mechanism. In contrast to what might have been expected in view of similarities between ACTH and MSH in some behavioural situations¹, comparable doses of ACTH₁₋₂₄ did not yield a clear-cut effect, but just a weak nigral reaction in half of the rats (Fig. 1b). The failure of ACTH₁₋₂₄ to affect the arcuate DA neurones is in keeping with the view that the DA system may be less important than noradrenergic neurones in the control of this hormone¹¹. But changes in tubero-infundibular DA neurones have been

Response of mesencephalic and hypothalamic dopamine neurones to α -MSH: mediated by area postrema?

α -MELANOTROPIN (α -MSH) influences mammalian behaviour in a variety of situations. It is released from the pars intermedia of the pituitary during stress and may have a physiological role in adaptive processes^{1,2}. In mammals, dopaminergic (DA) fibres of the tubero-hypophyseal system which originate in the arcuate nucleus of the hypothalamus, innervate the pars intermedia³; MSH secretion is usually inhibited by DA⁴. In rats, this DA system is subject to the influence of various extrahypothalamic brain regions thought to participate in the integration of endocrine and behavioural processes^{5,6}. The tubero-hypophyseal DA neurones may therefore occupy a key position between brain areas involved in effects of behaviourally active peptide hormones on one hand and the pars intermedia, a site of production of such peptides, on the other hand. We

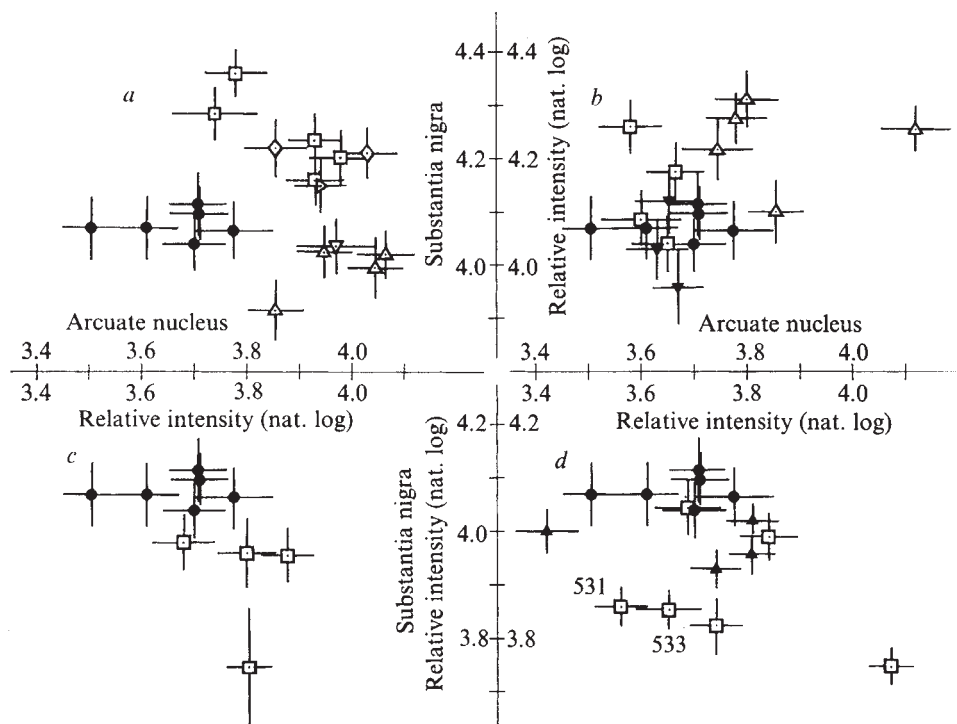


Fig. 1 Mean fluorescence intensity (bars = 95% confidence limits) of the DA neurone groups in the arcuate nucleus of the hypothalamus (abscissa) and in substantia nigra (ordinate) of individual rats. The saline-injected control group is shown for reference on each figure. *a*, Intensity 30 min (1 rat 15 min) after i.p. injection of α -MSH (Hoechst; dissolved in saline immediately before use, injection volume 0.1–0.2 ml per 100 g body wt.) at doses of ($\mu\text{g kg}^{-1}$) 100 ($\square$), 33 ($\diamond$), 10 ($\triangle$), 3 (∇), 10 (15 min; $\triangleright$) or saline ($\bullet$). *b*, Effect of ovine prolactin 2 mg per kg (Ferring; 1 mg per 0.25 ml solvent containing 1 mg phenol in 1 ml H_2O) ($\triangle$) or ACTH_{1-24} 175 μg per kg (Synacthen, CIBA; 0.25 mg ml^{-1}) ($\square$) administered i.p. Doses are approximately equimolar to the highest dose of α -MSH used. Saline shown as ($\bullet$); the phenol solvent itself remained without effect (∇). *c*, Blockade of the intensity change observed 30 min after α -MSH (100 $\mu\text{g kg}^{-1}$) by atropine 10 mg per kg injected 15 min before the peptide ($\square$). Saline only ($\bullet$). *d*, Mean fluorescence intensities in rats bearing lesions of area postrema as ob-

served 30 min after i.p. injection of 100 μg per kg α -MSH ($\square$) or saline ($\blacktriangle$). Saline in non-lesioned animals ($\bullet$). Numbers refer to the rats shown in Fig. 2. The values represent the combined right and left sides of the arcuate and nigral DA neurone groups; no differences were found between the two sides after lesions of area postrema. For each rat, the two brain pieces containing hypothalamus and midbrain were worked up together for microfluorimetry but investigated by different observers. The arcuate nucleus was studied at 15 antero-posterior levels (7- μm sections, distance between levels, 84 μm) starting at the anterior border of the external layer of the median eminence, the substantia nigra at 8 levels (distance between levels, 42 μm) starting at the anterior end of the fossa interpeduncularis^{4,6}. In the former all visibly fluorescent catecholamine-containing cells were measured on both sides at each of these levels (mean 215 ± 47 per rat), whereas in the latter 20 neighbouring cells per section were measured on one side starting at the dorsolateral corner of zona compacta (162 ± 13 per rat, except for lesioned rats where both sides were studied: 279 ± 41 per rat). The relative fluorescence intensity of a cell is defined by $i_r = (c - t)/(g_{\text{NA}} - g_t)$, c = absolute intensity of the cell, t = mean background fluorescence of neighbouring tissue, g_{NA} and g_t = mean intensities of noradrenaline-containing and noradrenaline-free standards of that section. For statistical analysis, the values were transformed into natural logarithms. Means of the transformed values are shown. Data were analysed by variance analysis followed by comparison of experimental groups with Scheffe's method of linear contrasts^{4,5}.

reported after manipulations of the pituitary adrenal axis¹². Our results do not exclude an action of ACTH_{1-24} on the two DA systems, but they indicate that this peptide was less effective than α -MSH in our experimental conditions.

The interpretation of central effects of peptides is complicated by the existence of the blood-brain barrier. Recent studies with labelled α -MSH suggest that the hormone may reach various brain regions, but its cellular localisation in brain tissue remains obscure¹³. The influence of α -MSH on extinction of a conditioned avoidance response is abolished by bilateral lesions in the parafascicular nuclei of the thalamus¹⁴. Although our

observations need not be related to this particular aspect of MSH action, it may be noted that both DA neurone groups reacted to electrical stimulation in this nucleus and in neighbouring areas⁶. These observations, together with results of lesions in the dorsomedial tegmentum of the lower brainstem⁶, seemed suggestive of a possible role of ascending projections. We focused our attention on the area postrema which is situated outside the blood-brain barrier¹⁵. Selective destruction of this area (Fig. 2) prevented the reaction of the arcuate DA neurones to α -MSH in four out of six rats (Fig. 1*d*, $P < 0.01$). In two rats with a partial response, we found intact tissue at the

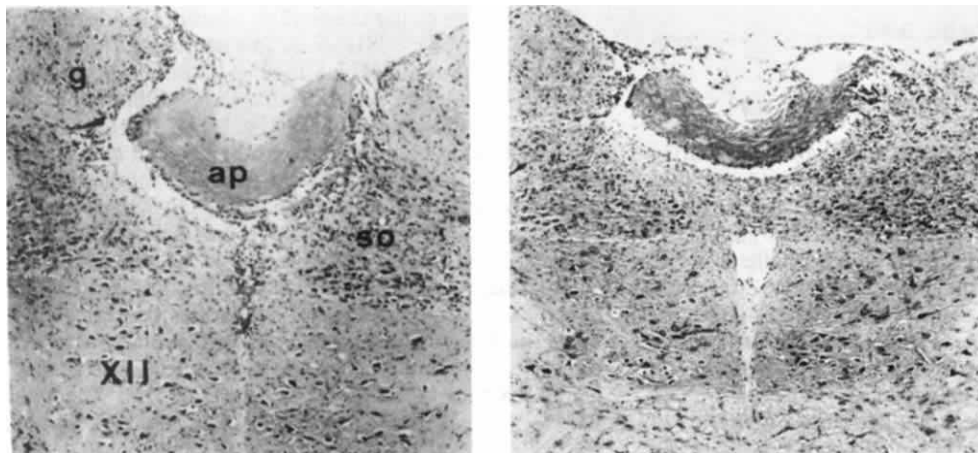


Fig. 2 Lesion of area postrema in rats nos 531 (left) and 533 (right). The lesion completely destroyed area postrema (ap) but impinged only slightly upon the dorsomedial corner of the nucleus of the solitary tract (so) and the medial edge of the nucleus gracilis (g). XII = nucleus nervi hypoglossi. Electrolytic lesions (250 μA , 60 s) were performed in gammabutyrolactone anaesthesia (750 mg kg^{-1} i.p., 1 h) according to coordinates calculated from various lesions in the lower brainstem, 2 weeks after ovariectomy. α -MSH was injected 5 d later between 15.00 and 16.00, after the usual 1-d steroid pretreatment. The rats recovered well from the operation and showed no neurological disturbances. They lost 6% of body weight, as did animals with ineffective electrode placements ($\times 49.2$).

posterior and anterior extremities of area postrema, respectively. In all rats, the reaction of the nigral DA neurones was not only blocked but apparently reversed, as mean intensity decreased to below the level of saline-injected intact rats ($P < 0.01$; saline-injected lesioned rats exhibited intermediary levels). Rats with electrode placements in the fourth ventricle in front of area postrema resulting only in a slight superficial damage to the ventricular wall, exhibited the usual reaction to α -MSH (mean intensity $\pm$ S.D. of these 3 rats (arcuate-nigral DA group): 3.965 ± 0.3966 ($n = 202$)/ 4.386 ± 0.3191 ($n = 240$); 3.837 ± 0.4470 (140)/ 4.282 ± 0.3570 (241); 3.987 ± 0.6252 (103)/ 4.354 ± 0.3561 (240).)

The effect of α -MSH thus seems to depend on the integrity of area postrema. It seems conceivable that α -MSH acted on this area, but a modulatory action of the peptide exerted at another brain level on an influence arising from area postrema, cannot be ruled out. The observations on the nigral DA neurones indicate that the effect of the lesion may be a complex one. The projection systems involved remain to be characterised. As atropine blocks responses of both DA neurone groups to electrical stimulation in various extrahypothalamic areas^{5,6}, we investigated its effect (Fig. 1c). Atropine completely prevented the intensity change in the nigral DA neurones ($P < 0.01$ against MSH without atropine). In the arcuate nucleus, the response was only partially reduced. Thus, cholinergic systems seem to be involved in the peptide effect, but the circuitry may differ in detail with respect to the two DA neurone groups.

We thank Miss R. Böswald and Mrs Z. Hefti for technical assistance. The study was supported by the Swiss NSF, the Hartmann-Müller Stiftung and the Jubiläumsspende of the University of Zürich.

W. LICHTENSTEIGER
R. LIENHART

Department of Pharmacology,
University of Zürich,
CH-8006 Zurich, Switzerland

Received November 30 1976; accepted February 25 1977.

- ¹ De Wied, D. *Frontiers in Neuroendocrinology* (eds Ganong, W. F. & Martini, L.) 97-140 (Oxford University Press, New York, 1969).
- ² Kastin, A. J. *et al. Progr. Brain Res.* 39, 461-470 (1973).
- ³ Björklund, A., Moore, R. Y., Nobin, A. & Stenevi, U. *Brain Res.* 51, 171-191 (1973).
- ⁴ Bower, A., Hadley, M. E. & Hruby, V. J. *Science* 184, 70-72 (1974).
- ⁵ Lichtensteiger, W. & Keller, P. J. *Brain Res.* 74, 279-303 (1974).
- ⁶ Lichtensteiger, W. & Lienhart, R. *Proc. Intern. Symp. cell. molec. Bases Neuroendocrine Processes* (ed. Endröczy, E.), 211-221 (Akadémiai Kiadó, Budapest, 1975).
- ⁷ Lichtensteiger, W., Felix, D., Lienhart, R. & Hefti, F. *Brain Res.* 117, 85-103 (1976).
- ⁸ Cotzias, G. C., Van Woert, M. H. & Schiffer, L. M. *New Engl. J. Med.* 276, 374-379 (1967).
- ⁹ Kostrzewa, R. M., Kastin, A. J. & Spirtes, M. A. *Pharmac. Biochem. Behav.* 3, 1017-1023 (1975).
- ¹⁰ Hökfelt, T. & Fuxe, K. *Neuroendocrinology* 9, 100-122 (1972).
- ¹¹ Scapagnini, U., Van Loon, G. R., Moberg, G. P., Preziosi, G. P. & Ganong, W. F. *Neuroendocrinology* 10, 155-160 (1972).
- ¹² Gouget, A., Duvernoy, J. & Bugnon, C. *C. r. Séanc. Soc. Biol.* 167, 919-923 (1973).
- ¹³ Kastin, A. J. *et al. Brain Res. Bull.* 1, 19-26 (1976).
- ¹⁴ Bohus, B. & De Wied, D. *Physiol. Behav.* 2, 221-223 (1967).
- ¹⁵ Wislocki, G. B. & Leduc, E. H. *J. comp. Neurol.* 96, 371-413 (1952).

Possible relationship between glial cells, dopamine and the effects of antipsychotic drugs

THE hypothesis that a functional excess of dopamine underlies many of the abnormalities seen in schizophrenia is receiving much attention. The supporting evidence is principally pharmacological. There is a correlation between antipsychotic drug action and the ability of a neuroleptic drug to block dopaminergic transmission¹⁻³. The exact site of antipsychotic drug activity is not known and evidence for the presynaptic inhibition of dopamine release⁴, as well as the blockade of postsynaptic receptors^{5,6} has been presented to explain neuroleptic drug action. Studies of binding properties of the dopamine receptor, however, provide a coherent explanation of neuroleptic drug action.

Butyrophenones, phenothiazines, thioxanines and diphenylbutylpiperadines inhibit ³H-haloperidol binding to a central nervous system (CNS) membrane fraction in exactly the order of their clinical potency⁷⁻¹⁰: the inhibition occurs at drug concentrations similar to those expected in the CNS of patients taking these drugs. These results with ³H-haloperidol, a dopamine antagonist, are used to argue that neuroleptics bind to postsynaptic dopamine receptors, and are effective by decreasing the activity of pathways using dopamine as their neurotransmitter. On the other hand, dopamine-sensitive adenylate cyclase is associated with the postsynaptic receptors^{5,11}, yet the inhibition of this enzyme by neuroleptic butyrophenones does not correlate with *in vivo* or clinical potency. The difference between butyrophenone inhibition of ³H-haloperidol binding and inhibition of adenylate cyclase has been attributed to "variable degrees of coupling of dopamine receptor sites with the adenylate cyclase"⁹. Alternatively, there could be several dopamine sensitive adenylate cyclases with differing binding affinities and localisations: this is suggested by the presence of this enzyme in several tissue culture lines of glia¹². Lesion studies show that the enzyme is not localised presynaptically in the caudate nucleus¹³ or in the substantia nigra¹⁴. The only defined dopaminergic receptors in the substantia nigra are localised on dopaminergic neurones¹⁵, and the elimination of these cells as the site of the enzyme was established by unilateral injections of 6-hydroxydopamine which removed these cells¹⁴. It therefore seems necessary to define more exactly the site of the dopamine binding receptor and the dopamine-stimulated adenylate cyclase. We report here that a large proportion of CNS dopamine haloperidol binding sites seem to be present on glial membranes and may be associated with an adenylate cyclase localised on these membranes.

We undertook to localise dopamine and haloperidol binding by fractionating rabbit cortex and bovine caudate and cortex. Synaptic fractions were prepared by the method of Gray and Whittaker¹⁶, and the P₂ pellet of this preparation was used as a cell-free CNS membrane fraction. Glial cell fractions were prepared¹⁷ and a C-6 cultured glial cell line was used for testing a clonal glial cell line. Binding assays were carried out in the presence of D and L butaclamol. Only the D isomer is active pharmacologically in blocking dopamine binding or as a clinical neuroleptic. Thus the difference between labelled dopamine and haloperidol binding in presence of the two isomers is taken as a measure of the blockade of specific dopamine receptors⁷⁻¹⁰. The assay used was essentially the centrifugation assay of Seeman *et al.*⁸. Although the filtration assay used by these workers and Snyder's group^{7,9} seemed to give similar results, we did not achieve the precision using this assay that was achieved using centrifugation and ¹⁴C-inulin to account for trapped external media in the pellet. Assay conditions were as in ref. 8 except that a different monoamine oxidase inhibitor (10.5 μ M pargyline-HCl) was added to buffer solutions.

Our initial results with fractions isolated from rabbit cortex showed some purification of dopamine binding in the membrane fraction (P₂ pellet) derived from CNS homogenate, but little subsequent purification when a synaptosomal fraction was isolated. This prompted us to look at the binding of ³H-dopamine and ³H-haloperidol on fractions isolated from beef caudate. The results in Table 1 clearly show that although there is some purification of the receptor in isolating the P₂ pellet, little further enrichment occurs when this fraction is subjected to density gradient centrifugation to isolate synaptosomes. After obtaining these results fractions enriched in astroglia were obtained from bovine caudate. An excellent glial cell fraction was isolated using the method for rabbit cortex with a slight modification

Table 1 Binding of ^3H -dopamine and ^3H -haloperidol, and activity of glutamic acid decarboxylase (GAD) in fractions isolated from the caudate nucleus of bovine brains

	Dopamine binding $\Delta\text{fmol per mg protein}$	Haloperidol binding $\Delta\text{fmol per mg protein}$	GAD activity $\mu\text{mol per mg per h}$
Homogenate	9.25 ± 3.4	3.9 ± 1.1	28.6
P ₂ fraction	17.65 ± 3.7	3.8 ± 0.9	54.8
Synaptosomal fraction	21.00 ± 3.5	6.5 ± 1.3	59.8
Glial fraction	35.05 ± 0.7	16.1 ± 0.9	4.0

Dopamine binding was measured with a final concentration of 1 nM ^3H -dopamine in the presence of 1 μM (+) or (–) butaclamol. Haloperidol binding was measured with 1 nM ^3H -haloperidol in the presence of 0.1 μM (+) or (–) butaclamol. The difference in binding between (+) butaclamol, a potent neuroleptic drug, and (–) butaclamol, an inactive drug, is taken as the stereospecific binding of the ligand. Binding assays represent triplicate determinations on at least two different glial cell preparations.

in the final Ficoll gradient¹⁷. The glia were collected between Ficoll solutions having refractive indices of 1.3633 and 1.3714, respectively. Binding studies in homogenates of these glial cells revealed a considerable purification of the receptor. This unexpected result suggests that the receptor which mediates neuroleptic drug activity is localised on glial membranes.

To assure that both dopamine and haloperidol binding take place on glial membranes we carried out several checks on the purity of our fractions. Morphological investigation by light and electron microscopy suggested that approximately 60% of the synaptosomal pellet is synaptic material, consistent with our previous findings on synaptic fraction purity¹⁸. The glial fractions also contain appreciable cell debris and, when viewed in the light microscope, 20% of the material seemed to be of non-glial origin. To quantitate this we assayed glutamic acid decarboxylase (GAD), the γ -aminobutyric acid (GABA) synthesising enzyme, in our fractions. The caudate is rich in GABA¹⁹ and GAD has been found almost exclusively in synaptic regions²⁰. The enzyme was assayed by the method of Roberts and Simonsen²¹. The results suggest that there is enrichment of synaptic material containing GAD in both P₂ and synaptosomal fractions while there is almost no GAD present in the glial fractions (Table 1). In addition, dopamine or haloperidol binding activity was found in a homogenate of C-6 glial cells, cells originating from a cloned line of rat glioma cells²². Furthermore, the glial dopamine-binding receptor seemed to be localised to regions of brain rich in dopamine in preliminary experiments.

To examine the possibility that glial dopamine sensitive adenylate cyclase could be associated with the binding site measured in either the ^3H -dopamine or ^3H -haloperidol assay, we examined the purification of the enzyme during cellular and subcellular fractionation. Cyclic 3'-5' AMP was measured in fractions using a modification of the protein binding assay of Gilman²³ (from Amersham Searle). The difference between dopamine-stimulated activity and basal activity was taken as a measure of the dopamine sensitive enzyme. The results of measurements on fractions from bovine caudate show that the enzyme activity is purified to approximately the same extent in glial cells as the binding site for ^3H -dopamine or ^3H -haloperidol. The enzyme showed 3.8-fold higher activity in the glial fraction than in the crude homogenate, and the binding of dopamine and haloperidol are similarly increased by fourfold. This finding, coupled with the results of Schubert *et al.*¹² demonstrating that clonal lines of glia contain a dopamine-stimulated adenylate cyclase and that in one glial line this enzyme was inhibited by haloperidol, suggests that the receptor in caudate could be an adenylate cyclase localised in glial cells.

The finding of a dopamine binding site on glial cells which may be associated with an adenylate cyclase suggests that a mechanism exists which would cause an increase in cyclic AMP in glia in response to neuronal activity. Such a metabolic response to neural release of neurotransmitter provides the initial step in what could be an important

feedback control regulating neuronal activity. The morphology of the synapse is consistent with such a control system, for synapses are surrounded by an astroglial sheath²⁴. Such a control system could involve changes mediated by cyclic AMP analogous to the increased glycogen breakdown seen in skeletal muscle²⁵ or membrane permeability changes as seen in smooth muscle²⁶, both mediated by a catecholamine-sensitive β -adrenergic receptor. Such an intercellular control provides a mechanism through which neuroleptic drugs could have a regionalised effect on CNS activity and dampen a wide variety of functions. This is an interesting possibility in view of the variety of symptoms seen in psychotic illness and the fact that neuroleptic drugs are neither specific nor curative in schizophrenia. The results of this preliminary study thus suggest that the receptor which correlates with the site of neuroleptic drug binding may reside on glial membranes and thus point to a possible mediation of neuronal activity by glial cells.

This work was supported by a NINCDS grant.

FRITZ A. HENN
DAVID J. ANDERSON
ÅKE SELLSTRÖM

Department of Psychiatry,
College of Medicine,
The University of Iowa,
500 Newton Road,
Iowa City, Iowa 52242

Received 23 November 1976; accepted 8 February 1977.

- 1 Van Rossen, J. M. *Archs int. Pharmacodyn. Théor* **160**, 492–494 (1966).
- 2 Matthysse, S. *Fedn Proc.* **32**, 200–204 (1973).
- 3 Synder, S. H., Banerjee, S. P., Yamamura, H. I. & Greengard, D. *Science* **184**, 1243 (1974).
- 4 Seeman, P. & Lee, T. *Science* **188**, 1217–1219 (1975).
- 5 Keibabian, J. W., Petzold, G. L. & Greengard, P. *Proc. natn. Acad. Sci. U.S.A.* **69**, 2145–2149 (1972).
- 6 Burt, D. R., Enna, S. J., Creese, I. & Synder, S. H. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4655–4659 (1975).
- 7 Creese, I., Burt, D. R. & Synder, S. H. *Life Sci.* **17**, 993–1002 (1975).
- 8 Seeman, P., Chau-Wong, M., Tedesco, J. & Wong, K. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4376–4380 (1975).
- 9 Creese, I., Burt, D. R. & Synder, S. H. *Science* **192**, 481–483 (1976).
- 10 Seeman, P., Lee, T., Chau-Wong, M. & Wong, K. *Nature* **261**, 717–718 (1976).
- 11 Iversen, L. K. *Science* **188**, 1084–1088 (1975).
- 12 Schubert, D., Tarikas, H. & La Corbiere, M. *Science* **192**, 471–472 (1976).
- 13 Mishra, R. K., Gardner, E. L., Katzman, R. & Makman, H. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3883–3887 (1974).
- 14 Keibabian, J. W. & Saavedra, J. M. *Science* **193**, 683–685 (1976).
- 15 Aghajanian, G. K. & Bunney, B. S. in *Frontiers in Catecholamine Research* (eds Usdin, E. & Synder, S.) 643 (Pergamon, New York, 1973).
- 16 Gray, E. G. & Whittaker, V. P. *J. Anat.* **96**, 79–89 (1962).
- 17 Henn, F. A. & Hamberger, A. *Neurochem. Res.* **1**, 261–273 (1976).
- 18 Henn, F. A., Anderson, D. J. & Rustad, D. G. *Brain Res.* **101**, 341–344 (1976).
- 19 Fahn, S. in *GABA in Nervous System Function* (eds Roberts, E., Chase, T. & Tower, D.) 172 (Raven, New York, 1976).
- 20 Wood, J. G., McLaughlin, B. & Vaughn, J. E. in *GABA in Nervous System Function* (eds Roberts, E., Chase, T. & Towers, D.) 143 (Raven, New York, 1976).
- 21 Roberts, E. & Simonsen, D. G. *Biochem. Pharmacol.* **12**, 113–134 (1963).
- 22 Pfeiffer, S. E., Herschman, H. R., Lightbody, J. & Sato, G. J. *Cell Physiol.* **75**, 329–340 (1970).
- 23 Gilman, A. G. *Proc. natn. Acad. Sci. U.S.A.* **67**, 305–312 (1970).
- 24 Bunge, R. P. in *The Neurosciences Second Study Program* (ed. Schmitt, O.) 782 (Rockefeller, New York, 1970).
- 25 Mayer, S. E. & Stull, J. T. *Ann N.Y. Acad. Sci.* **185**, 433–443 (1971).
- 26 Andersson, R., Lundholm, L., Mohn-Lundholm, E. & Nilsson, K. in *Adv. Cyclic Nucleotide Res.* (eds Greengard, P. & Robison, G. A.) 213–229 (Raven, New York, 1972).

Human prolactin responses to neuroleptic drugs correlate with antischizophrenic potency

THE secretion of prolactin, a pituitary hormone, is regulated by inhibitory and stimulatory influences from the hypothalamus. The primary influence is tonic inhibitory¹. Dopamine, a neurotransmitter, has a major direct and/or indirect role in mediating this inhibition upon the prolactin cells in the pituitary gland. This is documented by studies *in vitro*¹, *in vivo*² and in man³. All known neuroleptic, antischizophrenic drugs share one characteristic—they block dopaminergic transmission⁴⁻⁷. This action is hypothesised to be associated with their antischizophrenic effects (dopamine hypothesis)^{3,8}. Dopaminergic blockade is, very likely, also the primary mechanism of neuroleptics when inducing prolactin release via disinhibition^{1,10}. There is no evidence that neuroleptics release prolactin via stimulatory influences. We believe the prolactin response to neuroleptic drugs to be a valid and reliable model of dopaminergic blockade in man (Langer *et al.*, unpublished). Interest in the dopamine hypothesis of antischizophrenic drug action in man we postulated that comparing drug effects after acute intramuscular administration, a high correlation between prolactin releasing and antischizophrenic potencies of neuroleptics would be found.

Twelve healthy young male volunteers (medical students) were screened medically and psychiatrically for participation in the study and informed consent was obtained. On an average, each subject participated in seven weekly sessions, each starting at about 9 a.m., after an overnight fast, and lasting for 3½ h. At each session a single drug and dose was intramuscularly (i.m.) administered 30 min after a small angiograph had been inserted into a forearm vein of the recumbent subject. Throughout the study, blood samples were withdrawn at 15-min intervals into heparinised tubes. The plasma was immediately separated after the study and frozen at -20 °C. Prolactin was analysed in duplicate by radioimmunoassay¹¹. All samples from each subject were determined in one assay; variation between duplicate samples was 5.3%.

The following antischizophrenic drugs were selected because of their wide use in clinical psychiatry (which results in adequate documentation of their antischizophrenic effects) and because they are all intramuscularly administrable: haloperidol (0.25, 0.5, 1.0 and 1.5 mg), chlorpromazine (25.0 mg), prochlorperazine (2.5 and 5.0 mg), trifluoperazine (4.0 mg), perphenazine (1.0 mg), fluphenazine (0.35 mg), and thiothixene (0.25 and 0.5 mg). There is considerable interindividual variation of the prolactin response to a neuroleptic drug. We minimised the variation by (1) testing several drugs within the same subject, and (2) relating each subject's peak prolactin response to a neuroleptic to his own haloperidol dose-prolactin response curve. Haloperidol, a relatively specific dopamine receptor blocker and potent antischizophrenic drug, was chosen as reference drug for prolactin releasing and clinical potencies. So, all subjects were first tested with usually four different doses of haloperidol. Five of the subjects were then tested with two different doses of prochlorperazine and two different doses of thiothixene. We demonstrated that the dose-prolactin response curves to haloperidol, prochlorperazine and thiothixene, representing three different chemical classes of neuroleptics, were parallel (Langer *et al.*, in preparation). Eight of the subjects then received single doses of the various neuroleptics in doses which, according to a pilot study, induced about 50% of maximal prolactin release. To evaluate relative potencies, the peak prolactin response to a drug was related to the individual haloperidol dose-prolactin response curve. The potency per mg of each drug in releasing prolactin was then calculated in per cent potency of haloperidol in releasing prolactin. Finally, we related this value to the clinical potency of each drug relative to haloperidol. Data on antischizophrenic

potencies of drugs after acute parental administration were derived from standard clinical references^{12,13}.

Our results (Fig. 1) show that seven widely used antischizophrenic drugs show a very high correlation ($r = 0.956$) between their potency to release prolactin in man and their potency in the acute treatment of schizophrenic patients.

We thus hypothesise that mechanisms mediating these two different drug effects have at least one critical variable in common—the blockade of dopaminergic transmission. The parallelism of dose-prolactin response curves of the three representative drugs also indicates a common, dopaminergic, mechanism. We limit the interpretation of our findings to the

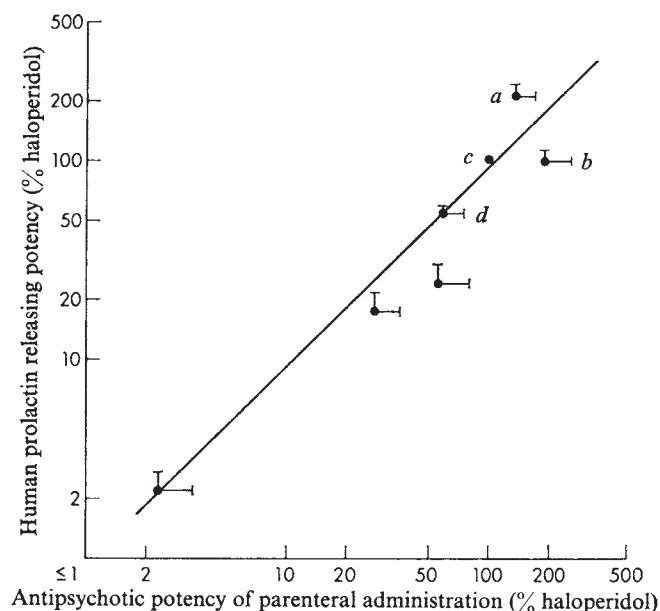


Fig. 1 The potency of seven antischizophrenic drugs in inducing prolactin release in man correlates with their potency in treating schizophrenic patients. Drugs tested (number of subjects in parentheses): a, thiothixene (6); b, fluphenazine (5); c, haloperidol (12); d, perphenazine (6); e, trifluoperazine (7); f, prochlorperazine (7); g, chlorpromazine (8). All values refer to an acute intramuscular administration and are expressed in per cent potency of haloperidol. Each value is the mean; vertical bars show the s.e.m. of prolactin values, horizontal bars the range of potency with conventional treatment. $y = 0.212 + 0.93x$, $r = 0.956$.

seven antischizophrenic drugs we tested, which constitute the majority of drugs used in the treatment of schizophrenia. So far, only one antischizophrenic, antidopaminergic drug (clozapine) shows inadequate prolactin-releasing activity. This might indicate that (1) increase of prolactin is not necessary for therapeutic response, and (2) different dopaminergic systems are primarily involved in prolactin-releasing and antischizophrenic effects. Prolactin release by neuroleptics probably reflects blockade of dopaminergic transmission at the hypothalamic and pituitary level, rather than at extrahypothalamic (possibly limbic) loci which are hypothesised to be associated with the therapeutic actions of antischizophrenic drugs¹⁴. It is noteworthy that schizophrenic patients generally require treatment of higher doses of a neuroleptic than are needed to achieve maximal prolactin release (1–2 mg haloperidol i.m. per 70 kg or dose equivalence of another neuroleptic drug).

This study supports in man the hypothesis that blockade of dopaminergic transmission is a characteristic of the neuroleptic drugs we tested and associated with their antischizophrenic effects. The questions whether the pathogenesis of schizophrenia specifically involves a disturbance in brain dopaminergic function and whether drugs necessarily have to interfere with

dopaminergic transmission to achieve antischizophrenic effects await the elucidation of the biological basis of schizophrenia.

This work was supported in part by a grant from the US Public Health Service. The National Pituitary Hormone Program (NIAMDD) is acknowledged for the gift of hPRL and antiserum to hPRL. We thank G. Huttinot, H. Novacenko and E. Stevens for their technical assistance.

GERHARD LANGER*
EDWARD J. SACHAR*
PETER H. GRUEN*
FRIEDA S. HALPERN*

Department of Psychiatry,
Albert Einstein College of Medicine,
Bronx, New York 10461

Received 20 December 1976; accepted 22 February 1977.

* Present address: Department of Psychiatry, College of Physicians and Surgeons of Columbia University, 722 West 168th Street, New York, New York 10032.

- 1 MacLeod, R. M. in *Frontiers of Neuroendocrinology* (eds Martini, L. & Ganong, W. F.) 169–194 (Raven, New York, 1976).
- 2 Lu, K. H. & Meites, J. *Endocrinology* **91**, 868–872 (1972).
- 3 Leblanc, H., Lachelin, G. C. L., Abu-Fadil, S. & Yen, S. S. C. *J. clin. Endocr. Metab.* **43**, 668–674 (1976).
- 4 Van Rossum, J. M. *Archs. Int. Pharmacodyn. Théor.* **160**, 492–501 (1966).
- 5 Horn, A. S. & Snyder, S. H. *Proc. natn. Acad. Sci. U.S.A.* **68**, 2325–2329 (1971).
- 6 Miller, R. J. & Iversen, L. L. *J. pharm. Pharmacol.* **26**, 142–147 (1974).
- 7 Clement-Cormier, Y. C., Kebejian, J. W., Petzold, G. L. & Greengard, P. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1113–1117 (1974).
- 8 Kety, S. S. & Matthysse, S. *Neurosci. Res. Prog. Bull.* **10**, 370–394 (1972).
- 9 Snyder, S. H., Banerjee, S. P., Yamamura, H. I. & Greenberg, D. *Science* **184**, 1243–1253 (1974).
- 10 Diefenbach, W. P., Carmel, P. W., Frantz, A. G. & Ferin, M. *J. clin. endocr. Metab.* **43**, 638–642 (1976).
- 11 Hwang, P., Guyda, H. & Friesen, H. *Proc. natn. Acad. Sci. U.S.A.* **68**, 1902–1906 (1971).
- 12 Byck, R. in *The Pharmacological Basis of Therapeutics* (eds Goodman, L. S. & Gilman, A. J.) 152–200 (Macmillan, New York, 1975).
- 13 Davis, J. M. *J. psychiat. Res.* **11**, 65–69 (1974).
- 14 Stevens, J. R. *Archs gen. Psychiat.* **29**, 177–189 (1973).

Defective dopaminergic regulation of prolactin secretion in a rat pituitary tumour cell line

It is firmly established that certain rat^{1,2} and human^{3,4} pituitary tumours hypersecrete prolactin (PRL). Pituitary PRL secretion is probably under the dual control of hypothalamic prolactin-inhibiting and -releasing factors, and defective secretion in one or both of these factors could produce abnormal PRL secretion³. An alternative explanation for the *in vivo* PRL secretory defect of certain pituitary tumours is defective reception by the tumour of an appropriate hypothalamic endocrine signal. To test this latter hypothesis, we evaluated the effects of various catecholamines on PRL secretion in a cloned rat pituitary tumour cell line (GH₃). Catecholamines are potent inhibitors of PRL secretion⁵, and it is postulated that the prolactin inhibiting factor may be a catecholamine^{6,7}. We report here that dopamine failed to inhibit PRL secretion from the pituitary tumour cultures, whereas, other catechols added to our normal and pituitary tumour monolayer cultures suppressed prolactin release into the medium. In addition, apomorphine, which is postulated to be a specific dopaminergic agonist^{8–10}, appropriately suppressed PRL secretion in the tumour monolayer cultures. Our results therefore provide evidence for an unusual dopaminergic defect in the GH₃ rat pituitary tumour cell line.

The pituitary tumour cell line was acquired from ATCC and was subcultured according to the method of Sato and Tasjian¹¹. Our normal rat pituitary monolayer cultures were produced by a modification of the method of Vale *et al.*¹² (Fig. 1). Rat PRL was measured by radioimmunoassay¹³.

Figure 1 illustrates that dopamine not only failed to suppress PRL secretion, but paradoxically stimulated PRL release in the pituitary tumour cell cultures. Significant PRL stimulation, however, was observed in most but not all of the individual experiments. Conversely, dopamine produced a significant suppression of PRL secretion in the preconfluent normal rat

monolayers. Other catecholamines added to the GH₃ cells and the normal rat pituitary cultures also produced suppression of PRL secretion.

The incubation conditions of our monolayer cultures for both the tumour and normal pituitary cells were similar (see Fig. 1), except that 15% horse and 2.5% foetal bovine serum were used with the tumour cells, and 20% foetal bovine serum was used in the normal rat monolayers. Dopamine is known to be susceptible to oxidant stress¹⁴ and we therefore had to demonstrate that the failure of dopamine to inhibit PRL secretion in the tumour cultures was not secondary to its rapid destruction by the horse serum. When dopamine was added to the normal pituitary cultures with 15% horse serum and 2.5% foetal bovine serum, PRL secretion was significantly inhibited, whereas dopamine added to the tumour cultures with 20% foetal bovine serum still failed to inhibit PRL secretion. Hence, failure of dopamine to inhibit prolactin secretion in the pituitary tumour monolayer cultures was not secondary to rapid destruction by the horse serum.

Another possible explanation for the lack of dopamine inhibition of PRL secretion was that it was a dose-related event. It has been suggested that adrenaline at low doses *in vitro* produced stimulation, and at high doses inhibition of PRL secretion¹⁵. To demonstrate that a biphasic effect was not present in the tumour cultures, PRL secretion was evaluated after addition of various concentrations of dopamine over a 40-fold range. In this experiment, each concentration of dopamine failed to significantly alter PRL secretion in the pituitary tumour cultures, whereas there was progressive inhibition of PRL secretion in the normal cultures of 25% by 1.5×10^{-8} M, 65% by 1.5×10^{-6} M, and 80% by 6×10^{-6} M dopamine. Hence, the PRL secretory defect was not explainable on the basis of the concentration of dopamine used in the experiments and was consistent with the presence of a dopaminergic defect in this pituitary tumour cell line. The observation, however, that apomorphine significantly suppressed PRL secretion in the tumour cultures (Fig. 1, Table 1) was difficult to reconcile with this conclusion. Apomorphine has been shown to be a dopaminergic agonist^{8–10} and the ability of pimozide, a specific dopaminergic blocking agent^{16–18} to inhibit the suppressive effect of apomorphine on PRL secretion in the tumour cultures (Table 1)

Table 1 The effect of a dopamine agonist and antagonist on pituitary tumour prolactin secretion.

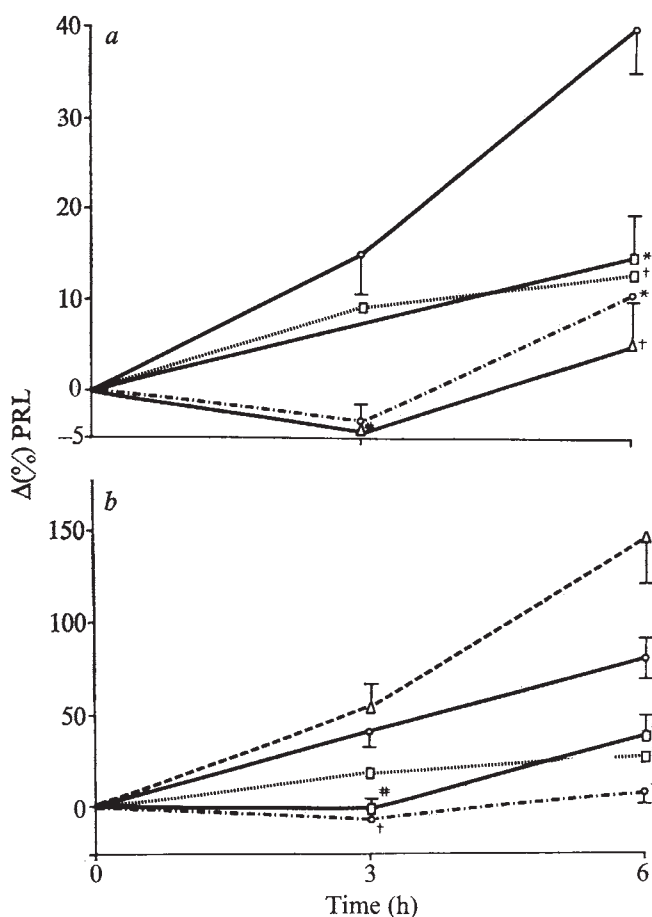
Treatment	n	Rat PRL (µg per well)	
		0 h	6 h
Controls	4	1.40 ± 0.09	1.89 ± 0.12
APO	5	1.16 ± 0.19	1.37 ± 0.11*
PIM	5	1.38 ± 0.09	1.79 ± 0.10
APO + PIM	5	1.53 ± 0.06	1.92 ± 0.04

Data are means ± s.e.m. APO, apomorphine 2.5×10^{-5} M; PIM, pimozide 4.0×10^{-6} M.

**P* < 0.02.

suggested that certain aspects of dopaminergic function were intact in the tumour cultures. Recent evidence has suggested that apomorphine may act only at presynaptic dopaminergic sites¹⁹, however, this finding does not explain our observations, as dopamine probably acts at both pre- and postsynaptic receptors¹⁹. It is possible therefore that the tumour cell line may have acquired a dopaminergic defect that permitted suppression of PRL secretion with apomorphine and yet failed to respond in a similar manner to dopamine. Alternatively, our data suggest that dopamine and apomorphine act at different sites in the rat pituitary. Hence defective dopaminergic regulation of prolactin secretion exists in this rat pituitary tumour cell line and may be related to the excessive prolactin secretion exhibited by certain rodent and human pituitary tumours.

This latter postulate, however, has to be viewed in the light of a large number of reports that indicate that L-dopa and/or its metabolite, dopamine, can inhibit rodent² and human^{3,4,20}



the same experiment. Vertical bars represent s.e.m. and Student's *t* test was used to evaluate the means with a *P* of < 0.05 being considered significant. Δ Represents results for dopamine 4×10^{-6} M ($n = 25$ in *a*; 34 in *b*); $\circ$ (on solid line) controls ($n = 41$ in *a*, 50 in *b*); $\circ$ (broken line), adrenaline 4.5×10^{-6} M ($n = 5$ in *a*, 9 in *b*); $\square$ (solid line), apomorphine 2.5×10^{-6} M ($n = 9$ in *a*, 13 in *b*); $\square$ (broken line), noradrenaline 4.5×10^{-6} M ($n = 13$ in *a*, 11 in *b*); significance levels shown as *, $P < 0.05$; †, $P < 0.002$; ‡, $P < 0.001$.

pituitary tumour PRL secretion. Interpretation of this *in vivo* data is complicated, however, by the observation that an adrenergic blockade can inhibit the effect of L-dopa in decreasing rat pituitary tumour PRL secretion². In addition, a recent report by Van Der Gugten *et al.*²¹ suggests that L-dopa decreased serum PRL concentrations in the rat in part by stimulating the peripheral uptake of PRL. Thus the reported suppressive effects of L-dopa on pituitary tumour PRL secretion may not be exerted via the dopaminergic receptor. Whether the dopaminergic defect described here is unique to this cloned cell line, or reflects a generalised defect in PRL secreting pituitary neoplasms remains to be established.

W. B. MALARKEY
J. C. GROSHONG

Department of Medicine,

G. E. MILO

Department of Physiological Chemistry,
Ohio State University College of Medicine,
Columbus, Ohio 43210

Received 27 December 1976; accepted 22 February 1977.

- ¹ Furth, J. Clifton, K. H., Gadsden, E. L. & Buffett, R. I. *Cancer Res.* **16**, 608–615 (1956).
- ² Malarkey, W. B. & Daughaday, W. H. *Endocrinology* **91**, 1314–1317 (1972).
- ³ Ganong, W. F. & Martina, L. (eds) *Frontiers in Neuroendocrinology* 337–374 (Oxford University Press, Oxford, 1973).
- ⁴ Malarkey, W. B. & Johnson, J. C. *Arch. Intern. Med.* **136**, 40–44 (1976).
- ⁵ Birge, C. A., Jacobs, L. S., Hammer, L. T. & Daughaday, W. H. *Endocrinology* **86**, 120–130 (1970).
- ⁶ MacLeod, R. M. & Lehmeyer, J. E. *Endocrinology* **94**, 1077–1085 (1972).
- ⁷ Sharr, C. J. & Clemens, J. A. *Endocrinology* **95**, 1202–1212 (1974).
- ⁸ Schoenfeld, R. & Uretsky, U. *Eur. J. Pharmac.* **19**, 115–118 (1972).
- ⁹ Rekker, R., Engel, D. & Nys, G. J. *pharm. Pharmac.* **24**, 589–591 (1972).
- ¹⁰ Smalstig, E. B., Sawyer, B. P. & Clemens, J. A. *Endocrinology* **95**, 123–129 (1974).

Fig. 1 The effect of catecholamines on PRL secretion in *a*, normal and *b*, tumour-bearing rat pituitary monolayer cultures. Normal cultures were prepared from anterior pituitaries removed from Holtzman male rats (200–300 g) immediately after killing by guillotine. Tissues were minced into 2-mm pieces with a sterile scalpel, and incubated for 45 min with gentle stirring at 37 °C with 0.5% collagenase (CIS Worthington) in 1X Detroit MEM with HEPES buffer at pH 7.2, supplemented with gentamycin (50 $\mu\text{g ml}^{-1}$), 1X amino acids (1 ml per 100 ml), 1 mM sodium pyruvate (1 ml per 100 ml), and 20% foetal calf serum (Grand Island Biochem.). The cell suspension was then centrifuged at 650g for 7 min. The supernatant was removed and the pellet resuspended with 10 ml of medium. This centrifugation and pellet resuspension in fresh medium was repeated once, and the final suspension was placed in 60 $\times$ 15 mm Linbro plates at a concentration of 3×10^4 to 7×10^4 cells per well to a final volume of 4 ml per well. All incubations were carried out at 37 °C (95% room air, 5% CO_2). The growth medium on these pre-confluent cultures was changed every 2–3 d and 10–24 h before the beginning of each experiment. At the beginning of an experiment, a 200- μl aliquot of the medium was removed from each well and stored at -20 °C for radioimmunoassay. The test substances were then added to each well and the progress of the experiments was followed by taking additional samples of the medium at 3 and 6 h. Rat PRL was measured by radioimmunoassay using RP-1 obtained from the NIAMDD as standard with tubes added to the assay to control for serum effects produced by the tissue culture medium. All of the catecholamines were prepared in the dark on ice immediately before addition to the culture to minimise oxidation. GH₃ cells (passage 20) were subcultured with trypsin (0.01 M) according to the methods of Sato and Tashjian⁵, and seeded in 60 $\times$ 15-mm Linbro plates at a concentration of 2×10^5 to 4×10^5 cells per well to a final volume of 4 ml per well. The culture medium consisted of Hams F-10 medium, supplemented with 2.5% foetal bovine serum, and 15% gamma-globulin-free horse serum (Grand Island Biochem.) and the pH was adjusted to pH 7.2. These pre-confluent cultures were allowed to grow for at least 72 h before study. After an experiment PRL secretion in each monolayer culture returned to its basal secretory rate. The normal and tumour figures represent pooled data from 8 and 9 separate experiments, respectively. PRL concentrations are expressed as % change from the basal PRL level. The data was transformed because of variations in basal PRL concentration of the individual wells between experiments and occasionally within the same experiment.

- ¹¹ Tashjian, A. H. Jr, Yasumura, Y., Levine, L., Sato, G. H. & Parker, M. L. *Endocrinology* **82**, 342–352 (1968).
- ¹² Vale, W., Blackwell, R., Grant, G., Guillemin, R. & Amoss, M. *Endocrinology* **91**, 562–572 (1972).
- ¹³ Niswender, G. D., Chen, C. L., Midgley, A. R., Meites, J. & Ellis, S. *Proc. Soc. exp. Biol. Med.* **130**, 793–796 (1969).
- ¹⁴ Takahara, J., Arimura, A. & Schally, A. *Endocrinology* **96**, 462–465 (1974).
- ¹⁵ Koch, Y., Lu, K. & Meites, J. *Endocrinology* **87**, 673–675 (1970).
- ¹⁶ Janssen, P. *et al. Arzneimittel-Forsch.* **18**, 261–279 (1968).
- ¹⁷ Anden, N. E., Butcher, S. G., Carrodi, H., Fuxe & Ungerstedt, V. *Eur. J. Pharmac.* **11**, 303–314 (1970).
- ¹⁸ Ojeda, S. R., Harms, P. G. & McCann, S. M. *Endocrinology* **95**, 1694–1703 (1974).
- ¹⁹ Dandya, P. C., Sharma, H. L., Patni, S. K. & Gambhir, R. S. *Experientia* **15**, 1441–1443 (1975).
- ²⁰ Malarkey, W. B., Jacobs, L. S. & Daughaday, W. H. *New Engl. J. Med.* **285**, 1160–1163 (1971).
- ²¹ Van Der Gugten, Sahuleka, P. C., Van Galen, G. H. & Kwa, H. G. *J. Endocr.* **68**, 369–381 (1976).

Prolactin–progesterone antagonism in self regulation of prolactin receptors in the mammary gland

THE role of prolactin during growth of the mammary gland and in lactation is well established (see ref. 1 for review) and the antagonising action of progesterone on lactogenesis (but not on mammary growth) has been recognised^{2–4}. A specific receptor for prolactin has been described⁵ and purified⁶ and it seems that the level of this receptor in the target cells is very sensitive to hormonal environments and might be one of the essential parameters which modulates the intensity of prolactin action. Titration of this receptor under controlled hormonal conditions has been mainly performed in the liver and its concentrations found to be sensitive to oestrogens⁷; this effect may be amplified or even mediated by prolactin itself⁸. We

have shown⁹ that the number of prolactin receptors in the mammary gland of pregnant or lactating rabbits undergoes a sudden increase at the onset of lactation but that the K_a of the receptor-hormone interaction remains constant. The question arises as to which hormonal environment is required for this amplification; is the inhibitory action of high progesterone levels on lactogenesis associated with a reduction of receptor concentrations? We describe here a study of this possibility and of the positive regulation of prolactin on its own receptors in the rabbit mammary gland. We extend to the mammary gland the stimulating effect of prolactin on the levels of its receptors and demonstrate an antagonising action of progesterone on this process.

New Zealand rabbits (4-6 months old) were used either in pseudopregnancy (mating with a vasectomised male) or at late pregnancy. Pseudopregnant animals were treated intramuscularly (i.m.) with 400 or 50 IU ovine prolactin (Byla) divided into four injections (two per d) over 48 h. Progesterone (Roussel) when administered was injected i.m. at a daily dose of 10 mg (two times 5 mg), starting the day before prolactin treatment. *In vivo* desaturation of receptor sites of their endogenous prolactin with bromocriptine (CB 154, Sandoz) was performed by injecting the drug subcutaneously (2 mg) 36, 24 and 12 h before the animals were killed, the first injection taking place 12 h after the last prolactin (and progesterone) treatment. Receptors were assayed as described before⁹ by estimating their total amount and their affinity

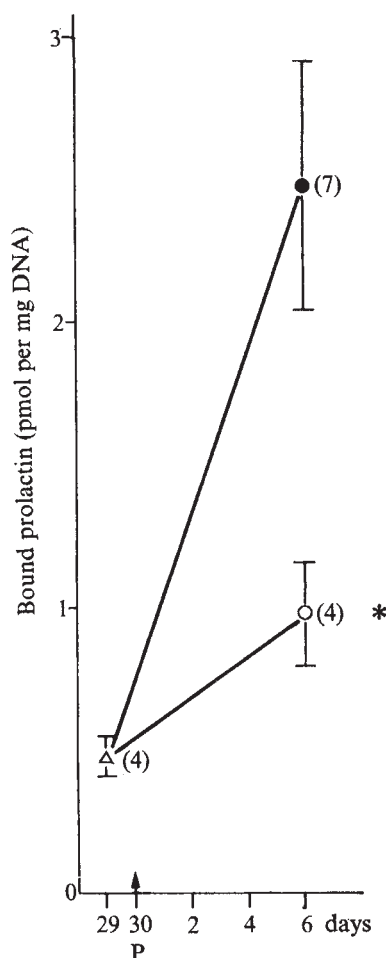


Fig. 1 Total number of prolactin receptors in the rabbit mammary gland titrated after 36 h *in vivo* desaturation. In the following conditions. △, Day 29 of pregnancy; ●, day 6 postpartum; ○, day 6 postpartum, prolactin surge at birth suppressed by ergocryptine (see text). Each point represents the mean $\pm$ s.e. for the number of animals indicated in parentheses. The affinity constant of the hormone-receptor interaction did not vary ($K_a = 3 \times 10^9 \text{ M}^{-1}$). *Significantly higher than control at the same day ($P < 0.01$).

constant according to the Scatchard plots¹⁰ obtained with varying concentrations of ovine prolactin labelled with ^{125}I and subtracting the non-specific binding. Protein concentrations were assayed according to Lowry *et al.*¹¹ and DNA according to Morimoto *et al.*¹².

Figure 1 shows that the total number of receptors per mg DNA in the mammary gland increases strikingly at parturition as shown previously⁹, but that this increase is inhibited by 60% ($P < 0.01$) when the prolactin surge at parturition is blocked by injecting CB 154 to the animals on the last day of pregnancy. In both cases, prolactin receptors were measured 6 d after parturition, since this delay corresponds to the highest levels normally observed. Interestingly the reduction in prolactin receptors was associated with a significant reduction in milk production (results not shown). This experiment is an illustration of the prominent role of prolactin inducing its own receptor at parturition, although it must be emphasised that this role probably requires a definite hormonal environment, since prolactin levels remain high in the rabbit at the onset of lactation, but seem unable at this period to act strongly on receptor concentrations. Figure 2 shows the effect of four doses of 12.5 IU and four of 100 IU of prolactin on the pseudopregnant rabbit and demonstrates the ability of the higher dose

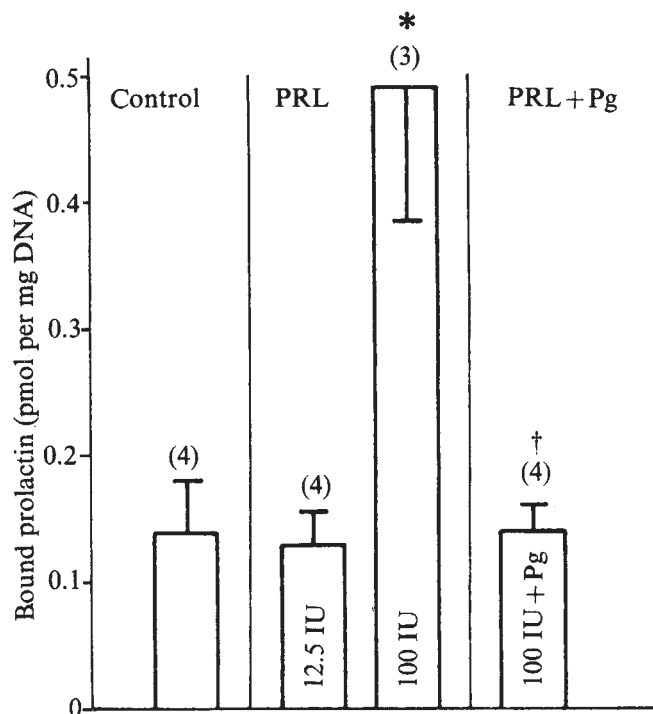


Fig. 2 Effect of prolactin (PRL) and prolactin+progesterone (PRL+Pg) injections on prolactin receptors concentrations in the mammary gland of 15 d pseudopregnant rabbits. Each point represents the means $\pm$ s.e. for the number of animals indicated in parentheses. The affinity constant ($K_a = 3 \times 10^9 \text{ M}^{-1}$) did not vary. *Significantly higher than control ($P < 0.025$). †Significantly lower than 100 IU prolactin treated animals ($P < 0.025$) and not different from control value.

of this hormone to increase its receptors in the mammary gland. The lower dose, though inactive when injected during 48 h, becomes effective when administered through more extended periods (results not shown). Progesterone injected simultaneously with a total dose of 400 IU of prolactin completely prevents any increase of the prolactin receptors in the mammary gland. This result extends the classical inhibitory effect of

progesterone on lactogenesis²⁻⁴ to an additional parameter—receptor concentration; it is not known whether this parameter is causally related to the various amplification mechanisms which ultimately cooperate in lactogenesis, by modulating the information available to the target cell, or whether it is just part of the pleiotypic action of prolactin on this cells protein synthesis.

It is thought that prolactin and progesterone receptors are both triggered initially by oestrogens^{7,13}, and hence might first appear in the mammary gland simultaneously with the oestradiol peak at the onset of pseudopregnancy. It is known that oestradiol induces its own receptors¹⁴ and that prolactin at least amplifies its own receptor levels in the liver⁸ and mammary gland (present results). In addition, progesterone counteracts both the self-regulated increase of oestradiol receptors¹⁵ and the increase of prolactin receptors in the mammary gland (present result). The interrelations between the circulating concentrations of these hormones and the levels of their receptors thus seem complex and it may be relevant to emphasise that the prolactin effect on the pseudopregnant rabbit (Fig. 2) might be increased if this hormone were injected at a period when luteolysis should already have begun, resulting in lower progesterone levels. This view is supported by the obvious requirement for a specific hormonal environment (high prolactin levels, a cortisol peak and decreasing progesterone levels) for the efficient induction of prolactin receptors at parturition (Fig. 1).

The mechanisms by which prolactin and progesterone regulate prolactin receptors are a matter of conjecture and it is doubtful that these hormones act at precisely the same level on the complex sequence of events which result in the synthesis, intracellular transport and possible removal of the receptors. The so-called down regulation (that is, lowering of receptor levels by its specific hormone) has been shown to be due to accelerated removal or breakdown of the hormone-receptor complex (possibly by endocytosis) at least in the case of insulin^{16,17}. This scheme should not apply to prolactin, which has just the opposite effect on its receptors. It remains to be shown to what extent this increase of receptors is due to an unmasking or recombination of previously inactive or segregated receptor molecules or to a *de novo* synthesis of these molecules.

We thank Professor H. Clauser for helpful advice. This work was supported by the Centre National de la Recherche Scientifique and the Délégation à la Recherche Scientifique et Technique

JEAN DJIANE
PHILIPPE DURAND

Laboratoire de Physiologie
de la Lactation,
Institut National
de la Recherche Agronomique,
78350 Jouy-en-Josas, France

Received 5 January; accepted 15 February 1977.

- 1 Denamur, R. J. *Dairy Res.* **38**, 237–264 (1971).
- 2 Kuhn, N. J. *J. Endocr.* **44**, 39–54 (1969).
- 3 Denamur, R. & Delouis, C. *Acta endocrin.* **70**, 603–618 (1972).
- 4 Assairi, L. et al. *Biochem. J.* **144**, 245–252 (1974).
- 5 Shiu, R. P. C. & Friesen, H. G. *Biochem. J.* **140**, 301–311 (1974).
- 6 Shiu, R. P. C. & Friesen, H. G. *J. biol. Chem.* **249**, 7902–7911 (1974).
- 7 Posner, B. I., Kelly, P. A. & Friesen, H. G. *Proc. natn. Acad. Sci. U.S.A.* **71**, 2407–2410 (1974).
- 8 Posner, B. I., Kelly, P. A. & Friesen, H. G. *Science* **188**, 57–59 (1975).
- 9 Djiane, J., Durand, P. & Kelly, P. A. *Endocrinology* (in the press).
- 10 Scatchard, G. *Ann. N.Y. Acad. Sci.* **51**, 660–672 (1949).
- 11 Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193**, 265–275 (1951).
- 12 Morimoto, H., Ferchmin, P. A. & Bennet, E. L. *Analyt. Biochem.* **62**, 436–448 (1974).
- 13 Reel, J. R. & Shih, Y. *Acta endocrin.* **80**, 344–354 (1975).
- 14 Gschwendt, M. & Kittstein, W. *Biochim. biophys. Acta* **361**, 85–96 (1974).
- 15 Hsueh, A. J., Peck, E. J., Jr. & Clark, J. H. *Nature* **254**, 337–339 (1975).
- 16 Kahn, C. R., Neville, D. M. & Roth, J. J. *J. biol. Chem.* **248**, 244–250 (1973).
- 17 Raff, M. *Nature* **259**, 265–266 (1976).

Alteration in glucocorticoid binding site number during the cell cycle in HeLa cells

GLUCOCORTICOID stimulation of alkaline phosphatase (AP) activity in HeLa cells is believed to be mediated by steroid receptors^{1,2}. When synchronised HeLa cells are exposed to glucocorticoids, an increase in AP activity is initiated during the synthesis phase of the cell cycle³. This 'S-phase dependency' suggests that the necessary components for cellular response to glucocorticoids are produced or altered during this time. Because the interaction of the steroid with its cytoplasmic receptor seems to be the first major intracellular event of the action of glucocorticoids, we have measured the glucocorticoid receptors present throughout the cell cycle in synchronised HeLa cells. We report here that although receptors are present throughout the cycle, the number per cell varies markedly within the cell cycle in both cytosols and whole cells.

HeLa S3 cells (adapted to suspension culture by Phillip Marcus, Albert Einstein Medical School) were maintained at a density between 2×10^5 and 6×10^5 cells per ml. The cells were synchronised by the double thymidine block procedure^{4,5}. Samples of cells for receptor analysis were collected from the synchronised cultures at late G₁, S, mitosis and early G₁. Scatchard analysis of glucocorticoid-binding in HeLa cell cytosols was performed using dextran-coated charcoal to remove free steroid. Uptake by whole cells was studied using a modification of the techniques described by Munck and Wira⁶.

As Fig. 1 shows, glucocorticoid receptors were present in cytoplasmic fractions at all stages of the cell cycle studied. Scatchard analyses of saturation curves indicate a single class of high affinity binding sites (Fig. 1). The affinities of the cytoplasmic receptors for dexamethasone did not vary by more than a factor of two and they agree with published results for dexamethasone-binding in other tissues¹¹. The number of cytoplasmic dexamethasone-binding sites per cell doubled between late G₁ and S (Fig. 1). Cells collected at S showed maximal rates of incorporation of ³H-thymidine. Between mid-S-phase and mitosis (4 h) there was a considerable reduction in cytoplasmic dexamethasone-binding per cell, which correlated with a doubling of cell number. By early G₁ there had been a small increase in binding. We have observed this pattern of alteration in numbers of glucocorticoid receptors in seven identical synchrony experiments. The data suggest that the number of cytoplasmic glucocorticoid receptors per cell doubles before mitosis, with less than half the complement available for assay in the daughter cells.

When the number of glucocorticoid-binding sites is expressed as a function of other biochemical parameters (DNA, protein and cell volume) (Table 1), a similar, cell cycle-dependent variation is seen, although it is less pronounced. This variation was also observed in all seven experiments.

We have assessed dexamethasone-binding after incubation of whole cells with the steroid at both 3 and 37 °C. Figure 2 shows the results of one of four nuclear translocation experiments performed on cells from separate synchronised populations.

As in studies of ³H-dexamethasone binding in cytosols, the level of saturable ³H-dexamethasone-binding in whole cells showed cell cycle dependence. Figure 2a shows that, as in other systems¹¹ most glucocorticoid-binding was restricted to the cytoplasmic fractions after incubation at 3 °C, with very little binding associated with the nucleus. Cytoplasmic binding in whole cells at 3 °C significantly increased between late G₁ and synthesis and decreased to $\approx 1/2$ synthesis values after mitosis. Nuclear binding at 3 °C did not show similar cell cycle dependence. When cells were warmed to 37 °C (Fig. 2b) there was a significant increase in nuclear binding at each stage of the cell cycle, with a concomitant reduction in the level of cytoplasmic binding. Both cytoplasmic and nuclear binding at 37 °C showed the cell cycle dependence observed with cytosol at 3 °C (Fig. 1) and whole cell cytoplasmic fractions at 3 °C (Fig. 2a). Total-cell binding (cytoplasmic and nuclear) was similar when measured at 3 °C (Fig. 2a) or at 37 °C after 2 h at 3 °C (Fig.

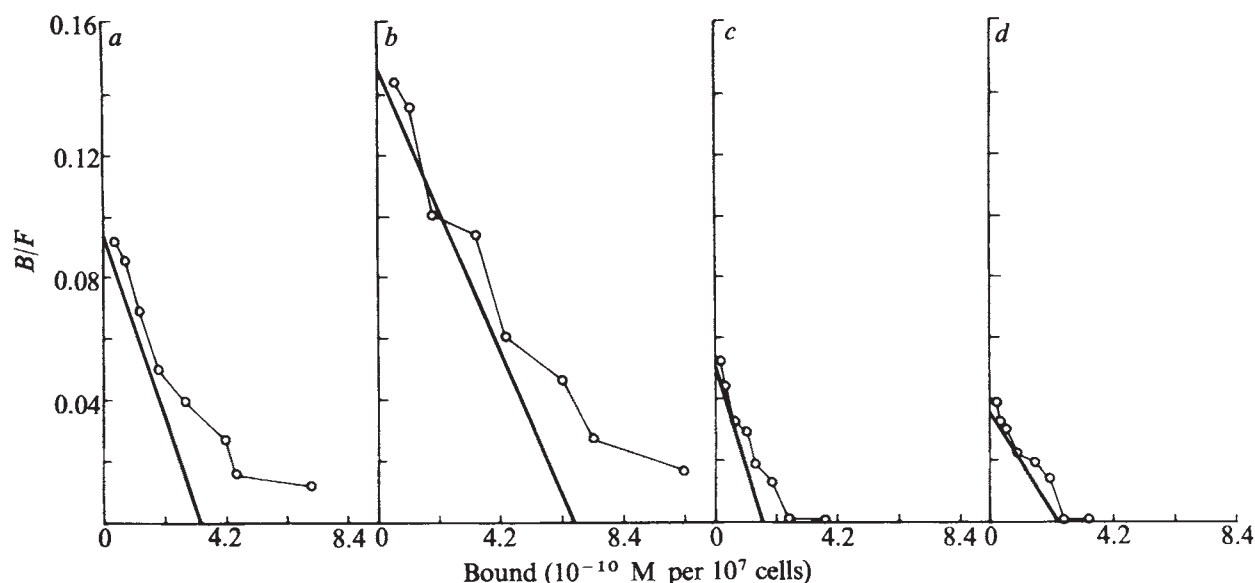


Fig. 1 Scatchard analysis of glucocorticoid-binding in HeLa cell cytosols. HeLa cells were grown in Joklik's modified Eagle's medium supplemented with 3.5% each of calf and foetal calf serum in the presence of kanamycin ($10 \mu\text{g ml}^{-1}$) in suspension culture at 37°C . The cells have a doubling time of about 20 h. The cells were synchronised by double thymidine block^{4,5}. They were grown in 2 mM thymidine for 16 h, followed by growth for 10 h in thymidine-free medium. Thymidine treatment was repeated for a further 14 h and the cells were washed free of thymidine. Growth was then resumed in thymidine-free medium; time of release from the second thymidine block is time 0. During late G_1 S, mitosis and early G_1 , 200-ml samples of cells were collected and centrifuged at 600g for 5 min and the resulting pellet was resuspended in 1.5 ml of medium containing 10% glycerol. After a period of slow cooling, cells were kept frozen in liquid nitrogen. At the time of assay, cells were thawed at 23°C and washed with 50 ml of serum-free medium. Freezing and thawing had no effect on receptor affinity and binding sites per cell when tested with randomly growing cells. The thawed HeLa cells were centrifuged and resuspended in 2.5 ml Joklik's medium (without serum) at room temperature and three 200- μl samples were removed for determination of cell numbers, DNA⁷ and total cell protein⁸. The cells were then pelleted by centrifugation at 600g, the medium was aspirated off and 3.0 ml of ice-cold Tris-EDTA buffer (0.01 M Tris, 0.0015 M Na_2EDTA pH 8.0) was added to each pellet. Cell pellets were homogenised at $0-3^\circ\text{C}$ with three 5-s bursts from a Polytron (Brinkman Instruments) at setting 5. All subsequent procedures were performed at 3°C . The homogenates were centrifuged at 12,500g for 4 min in an Eppendorf model 5411 microfuge. The supernatants (cytosol) were decanted into glass tubes and divided into 50- μl samples of eight different concentrations of ^3H -dexamethasone (prepared in 0.9% NaCl) from 1×10^{-7} M to 1×10^{-10} M. The cytosols were incubated for 90 min at 3°C . This time was sufficient to attain near-maximal dexamethasone-binding in cytosol from randomly growing HeLa cells, in mammary gland cytosol⁹ and in muscle cytosol¹⁰. After this incubation, 100- μl samples of dextran-coated charcoal (1% Norit A and 0.1% dextran type 60 C in 1.5 mM MgCl_2) was added to each sample. The tubes were then vortexed and left standing to incubate for 15 min, and subsequently centrifuged at 12,500g for 2 min. Samples of 75 μl of the supernatant were placed into 5 ml of Bray's solution for determination of radioactivity. Duplicate 5- μl samples of the original ^3H -dexamethasone solutions used for incubation with cytosol were counted for determination of total steroid concentrations. The percentage of cells in mitosis was determined by monitoring a parallel culture of synchronised cells growing in the presence of colchicine ($0.2 \mu\text{g ml}^{-1}$). Cells from growing culture were counted microscopically in a haemocytometer. Radioactivity was determined in a Packard (3310) liquid scintillation spectrometer with a 25% efficiency for tritium. Each cytosol preparation was analysed for protein by the method of Lowry⁹. DNA was measured by a microfluorometric assay¹⁰. The cytocrit of each cell suspension was determined using a Drummond microhaematocrit, a, Late G_1 = 24,802 sites per cell; b, synthesis = 46,950 sites per cell; c, mitosis = 12,040 sites per cell; d, early G_1 = 16,615 sites per cell. The K dissociations ranged from 1.3×10^{-8} M to 2.6×10^{-8} M.

2b), indicating that equilibrium was probably attained within 2 h at 3°C during each stage of the cell cycle.

Our results show that glucocorticoid-binding occurs in HeLa S3 cells throughout the cell cycle when measured in cytosols and in cytoplasmic and nuclear fractions of whole cells. The 'S-phase dependency' of stimulation of AP activity therefore cannot be attributed to an absence of glucocorticoid receptors or the failure of the steroid receptor complex to be translocated to the nucleus at other stages of the cell cycle. Although it is possible that a critical number of receptors is needed for AP stimulation, other factors are more probably involved, since some cells with fewer binding sites are responsive to glucocorticoid stimulation¹¹. Of the factors that involve the receptor, the following are likely candidates: (1) modification of the receptor itself (not at the binding site); (2) lack

of functional nuclear acceptor sites, or (3) an altered nuclear processing of the steroid receptor complex. These can all show cell cycle dependence.

We have demonstrated that in the conditions we used to synchronise HeLa cells, the number of cytoplasmic and nuclear dexamethasone-binding sites is regulated in a specific manner in relation to the cell cycle. The simplest interpretation of the observed doubling of the number of receptors at S is that this represents the synthesis of new receptor for passage to daughter cells. Histone synthesis at S is an example of a regulatory component that is synthesised at one phase of the cell cycle¹². The increased number of receptors, however, may not result from the synthesis of new receptors but could reflect activation of a proreceptor, differential degradation of receptor molecules or

Table 1 Relation of cell cycle stage to cytoplasmic receptor concentration per unit of protein, DNA and cell volume

Cell cycle stage	Late G_1	S	Mitosis	Early G_1
Moles bound				
per 100 mg total protein	1.44×10^{-13}	2.0×10^{-13}	1.0×10^{-13}	1.70×10^{-13}
per 100 mg cytosol protein	1.45×10^{-13}	2.1×10^{-13}	1.5×10^{-13}	2.15×10^{-13}
per 100 μg DNA	1.42×10^{-13}	1.71×10^{-13}	0.68×10^{-13}	0.77×10^{-13}
per ml of packed cells	20×10^{-13}	24×10^{-13}	12×10^{-13}	22×10^{-13}

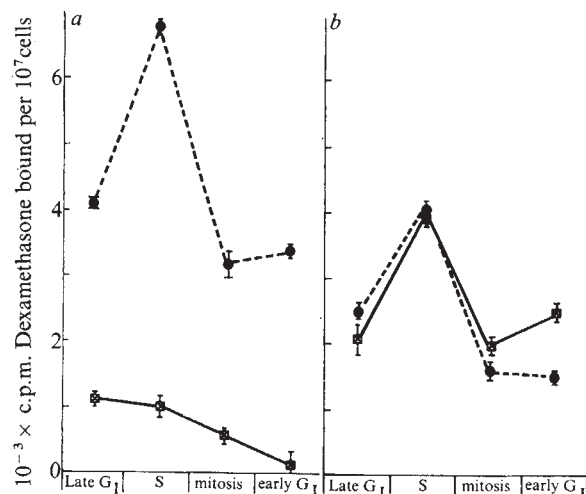


Fig. 2 Saturable cytoplasmic and nuclear ^3H -dexamethasone-binding in synchronized HeLa cells after incubation of whole cells with steroid. The thawed HeLa cells were suspended in 1.0 ml of ice-cold Joklik's medium without serum. Samples of 400 μl of cell suspensions from each stage of the cell cycle were incubated for 2 h at 3 $^{\circ}\text{C}$ (a) or 2 h at 3 $^{\circ}\text{C}$ plus 30 min at 37 $^{\circ}\text{C}$ (b) with $\approx 1 \times 10^{-8}$ M ^3H -dexamethasone alone or ^3H -dexamethasone plus 1×10^{-6} M unlabelled dexamethasone. Saturable dexamethasone-binding was considered to be the difference between the observed binding with ^3H -dexamethasone alone and ^3H -dexamethasone plus unlabelled dexamethasone. Cytoplasmic ($\bullet$) and nuclear ($\square$) binding were measured in 20- μl samples of cells after cell lysis in dextran-coated charcoal (cytoplasmic) or 1.5 mM MgCl_2 (nuclear) as before⁶. A rapid freeze-thaw modification was used to ensure breakage of the cells. The values shown are the means $\pm$ s.e. of triplicate determinations of the saturable binding fraction from a single synchrony experiment.

masking or unmasking of the binding sites. If the doubling of receptor number between late G_1 and S represents synthesis of new receptors, these cells may provide an appropriate system for the isolation of receptor mRNA.

We thank Professor A Munck for suggestions and criticism and Sally Whitlock for technical assistance. This project was supported in part by grants from NCI, NIAMDD and NIH.

JOHN A. CIDLOWSKI

Department of Physiology

GEORGE A. MICHAELS

Department of Microbiology,
Dartmouth Medical School,
Hanover, New Hampshire 03755

Received 12 October 1976; accepted 18 January 1977.

¹ Melnykovich, G. & Bishop, C. *Biochim. biophys. Acta* **177**, 579-585 (1969).

² Melnykovich, G. & Bishop, C. *Endocrinology* **88**, 450-455 (1971).

³ Griffin, M. J. & Ber, R. *J. Cell Biol.* **40**, 297-304 (1969).

⁴ Bottsma, D., Budke, L. & Vos, O. *Exp. Cell Res.* **33**, 301-309 (1964).

⁵ Puck, T. T. *Science* **144**, 565-566 (1964).

⁶ Munck, A. & Wira, C. in *Methods in Enzymology* (eds O'Malley, B. & Hardman, J.) (Academic, New York, 1975).

⁷ Zubroff, J. & Sarma, D. S. R. *Analyt. Biochem.* **70**, 387-396 (1976).

⁸ Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193**, 265-275 (1951).

⁹ Shymala, G. *Biochemistry* **12**, 3085-3089 (1973).

¹⁰ Mayer, M., Kaiser, N., Milholland, R. J. & Rosen, F. *J. biol. Chem.* **249**, 5236-5240 (1974).

¹¹ Munck, A. & Leung, K. in *Receptors and Mechanism of Action of Steroid Hormones* (ed Pasqualini, J. R.) (Dekker, New York, in the press).

¹² Robbins, E. & Borun, T. W. *Proc. natn. Acad. Sci. U.S.A.* **57**, 409-416 (1967).

these tumour-bearing animals^{2,5-8}. A similar hypercalcaemic syndrome occurs in some patients with cancer, is associated with excessive PGE_2 metabolite excretion in urine and is reversed at least in part by treatment with aspirin-like drugs^{9,10}. Finally, intravenous infusion of PGE_2 in intact, unanaesthetised rats causes an increase of plasma calcium concentration¹¹. The most straightforward interpretation of these findings is that PGE_2 is secreted by certain tumour cells into plasma in such quantity that the steady-state plasma concentration of PGE_2 is sufficiently high to stimulate osseous target cells to resorb bone. An alternative interpretation is that unmeasured intermediates in arachidonic acid metabolism or PGE_2 metabolites have sufficient bone resorption-stimulating activity to be the agent acting on bone cells. This hypothesis gains a degree of support from knowledge that some prostaglandin metabolites have longer half lives in plasma than PGE_2 itself¹². We have now measured the biological activities of several endoperoxide analogues and PGE_2 metabolites in a bone culture assay system and compared their potencies with that of PGE_2 .

The bone resorption-stimulating activities of three prostaglandin endoperoxide analogues are shown in Table 1. Azo-PG III, which stimulates platelet aggregation, release of 5-hydroxytryptamine and contraction of isolated rabbit aorta¹³, did not stimulate bone resorption at doses up to 10 $\mu\text{g ml}^{-1}$. The other two analogues were only active;

Table 1 Effects of prostaglandin endoperoxide analogues U-44069 U-46619 and azo-PG III on bone resorption

Experiment	Treatment	Dose ($\mu\text{g ml}^{-1}$)	Medium calcium (mg per 100 ml)
1	None	—	7.6 $\pm$ 0.43
	PGE_2	0.005	8.6 $\pm$ 0.43
	PGE_2	0.025	12.1 $\pm$ 0.43*
	U-44069	4	8.1 $\pm$ 0.43
	U-44069	20	11.4 $\pm$ 0.43*
2	None	—	7.6 $\pm$ 0.36
	PGE_2	0.004	9.1 $\pm$ 0.36†
	PGE_2	0.050	13.8 $\pm$ 0.36*
	U-44069	10	10.1 $\pm$ 0.36*
	U-46619	10	8.6 $\pm$ 0.36‡
3	None	—	7.0 $\pm$ 0.43
	PGE_2	0.05	12.4 $\pm$ 0.38*
	U-44069	15	11.0 $\pm$ 0.38*
	U-46619	15	8.4 $\pm$ 0.38‡
4	None	—	6.8 $\pm$ 0.50
	PGE_2	0.01	8.8 $\pm$ 0.50†
	PGE_2	0.10	10.3 $\pm$ 0.50*
	Azo-PG III	5	6.8 $\pm$ 0.50
	Azo-PG III	1	5.7 $\pm$ 0.50
5	None	—	6.2 $\pm$ 0.46
	PGE_2	0.01	9.1 $\pm$ 0.46*
	PGE_2	0.10	10.6 $\pm$ 0.46*
	Azo-PG III	10	6.4 $\pm$ 0.46

Bone resorption was determined by measuring the release of calcium (^{45}Ca) from neonatal mouse calvaria in organ culture^{2, 14}. Medium total calcium concentration was measured with a Corning model 940 calcium analyser. Medium was Dulbecco's modified Eagle's medium supplemented either with bovine serum albumin (5 mg ml^{-1}) or heat-inactivated (60 $^{\circ}\text{C}$), 1 h horse serum (15% final concentration) and foetal calf serum (2.5% final concentration). Results were essentially the same in both kinds of medium. Treatment groups consisted of three or four bones each. Results given are mean values for medium total calcium $\pm$ s.e. at the end of 48 h of treatment with the compounds tested. U-44069 is (15S)-hydroxy-9 α ,11 α -(epoxymethano)prosta-5Z,13E-dienoic acid (lot no. 12344-JMB-85). U-46619 is (15S)-hydroxy-11 α ,9 α -(epoxymethano)prosta-5Z,13E-dienoic acid (lot no. 12344-JMB-109). Azo-PG III is (5Z, 9 α , 11 α , 13E, 15S)-9,11-azo-15-hydroxyprosta-5,13-dienoic acid¹³. The data in each experiment were subjected to an analysis of variance and s.e. was calculated from the residual error form of that analysis.

*Statistical difference from control mean $P < 0.001$.

†Statistical difference from control mean $P < 0.01$.

‡Statistical difference from control mean $P < 0.05$.

Biological activities of prostaglandin analogues and metabolites on bone in organ culture

PROSTAGLANDINS, especially the E-series, are potent stimulators of bone resorption in several *in vitro* systems¹⁻⁴. Studies with two experimental tumours (HSDM₁ mouse fibrosarcoma and VX₂ rabbit carcinoma) have indicated that excessive synthesis and secretion of prostaglandin E₂ (PGE_2) by the neoplastic cells is associated with and seems likely to be responsible for the hypercalcaemia observed in

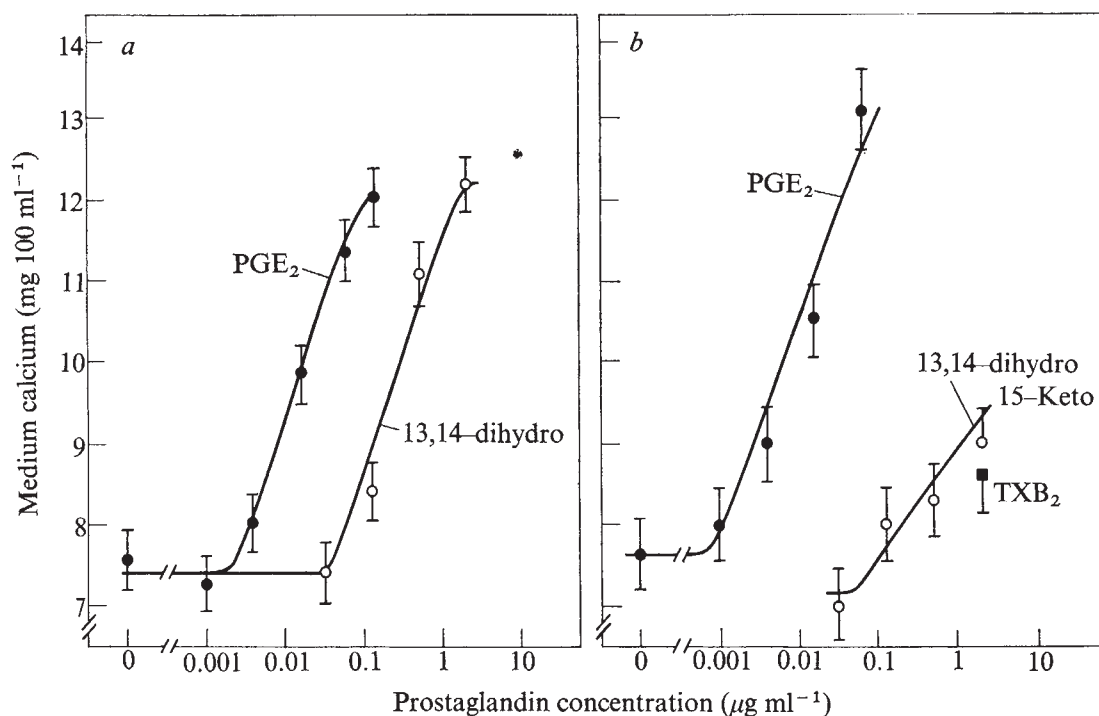


Fig. 1 Effects of increasing concentrations of PGE₂, 13,14-dihydro-PGE₂ and 13,14-dihydro-15-keto-PGE₂ on bone resorption in organ culture. Neonatal mouse calvaria were cultured and analysed as described in Table 1 except that both experiments were performed in heat-inactivated serum medium. Furthermore, the calvaria in these experiments were free floating instead of attached to cover slips as described in the original procedure^{2, 14}. This modification simplifies the procedure without affecting the outcome. Each point gives the mean value for groups of three or four bones, and the vertical bars give the s.e. at the end of 48 h of treatment. The s.e. were calculated from an analysis of variance. *a*, PGE₂ (●); 13,14-dihydro-PGE₂ lot no. 12874-JHK-52A (○). *b*, PGE₂ (●); 13,14-dihydro-15-keto-PGE₂, lot no. 12277-JHK-122A (○); thromboxane B₂, lot no. 13066-RWJ-38A (■).

U-44069 and U-46619 were about 0.08% and 0.06%, respectively, as active PGE₂. None of these analogues was an effective antagonist of PGE₂-stimulated bone resorption (data not shown).

The bone absorption-stimulating activities of the PGE₂ metabolites 13,14-dihydro-PGE₂ and 13,14-dihydro-15-keto-PGE₂ were about 6.6% and 0.20%, respectively, as active as PGE₂ (Fig. 1). Thromboxane B₂ had even less biological activity than 13,14-dihydro-15-keto-PGE₂.

Our results indicate that the bone resorption-stimulating activity of PGE₂ is at least 1,000 times greater than that of the three prostaglandin endoperoxide analogues tested. PGE₂ is also about 15 times more potent than 13,14-dihydro-PGE₂ and about 500 times more potent than 13,14-dihydro-15-keto-PGE₂, although an accurate estimate of the potency of the latter metabolite was not possible because its dose-response curve was not parallel to that of PGE₂ (Fig. 1).

Because of their relatively low biological activities, if any of the metabolites were responsible for the bone resorption and hypercalcaemia seen in HSDM₁ and VX₂ tumour-bearing animals, the half lives of these compounds in plasma would need to be considerably greater than that of PGE₂, thus making it possible for them to accumulate to very high levels in plasma and extracellular fluid. The half life of PGE₂ is very short, probably less than 1 min¹². The half life of native prostaglandin endoperoxides in aqueous media is about 5 min¹² and probably less in plasma although this has not been determined accurately. The half life in plasma of 13,14-dihydro-15-keto-PGE₂ is about 8 min and that of 13,14-dihydro-PGE₂ is not known¹². Of the compounds tested, the most likely mediator of bone resorption, other than PGE₂, would be 13,14-dihydro-PGE₂ because its biological activity was the highest, about 6-7% of PGE₂. Unfortunately, no data are available on plasma concentrations of 13,14-dihydro-PGE₂ in control or tumour-bearing animals because there are no assays

for this metabolite. We have reported that in tumour-bearing mice, plasma concentrations of 13,14-dihydro-15-keto-PGE₂ are considerably higher than corresponding levels of PGE₂⁸. This increment was not sufficiently large to cause hypercalcaemia in the mice because of the very low bone resorption-stimulating activity of 13,14-dihydro-15-keto-PGE₂. Seyberth *et al.* have also noted a greater increase in plasma 13,14-dihydro-15-keto-PGE₂ than PGE₂ in rabbits bearing the VX₂ carcinoma¹³, and L. G. Raisz has observed (personal communication), using foetal rat long bones in culture, that 13,14-dihydro-PGE₂ and 13,14-dihydro-15-keto-PGE₂ are substantially less active stimulators of bone resorption than PGE₂.

Although our data do not rule out the possibility that prostaglandin endoperoxides or metabolites are the mediators of bone resorption and hypercalcaemia in the HSDM₁ and VX₂ model systems, we believe that the data available strongly suggest that PGE₂ is the mediator. Measurements of 13,14-dihydro-PGE₂ in plasma will provide more evidence when they are available.

Prostaglandins, endoperoxides and metabolites were given by Dr Udo Axen of the Upjohn Co., with the exception of azo-PG III which was supplied by Professor E. J. Corey of Harvard University. We thank Mrs E. A. Moore for statistical calculations. This investigation was supported in part by the USPHS.

ARMEN H. TASHJIAN, JR
JANET E. TICE
KAREN SIDES

Harvard School of Dental Medicine,
Harvard Medical School,
Boston, Massachusetts 02115

Received 20 December 1976; accepted 21 January 1977.

¹ Klein, D. C. & Raisz, L. G. *Endocrinology* 86, 1436-1440 (1970).

² Tashjian, A. H., Jr, Voelkel, E. F., Levine, L. & Goldhaber, P. *J. exp. Med.*, 136, 1329-1343 (1972).

³ Dietrich, J. W., Goodson, J. M. & Raisz, L. G. *Prostaglandins* 10, 231-240 (1975).

- ⁴ Powles, T. J., Easty, D. M., Easty, G. C., Bondy, P. K. & Munro-Neville, A. *Nature new Biol.* 245, 83–84 (1973).
- ⁵ Tashjian, A. H., Jr, Voelkel, E. F., Goldhaber, P. & Levine, L. *Fedn Proc.* 33, 81–86 (1974).
- ⁶ Tashjian, A. H., Jr, Voelkel, E. F., Goldhaber, P. & Levine, L. *Prostaglandins* 3, 515–524 (1973).
- ⁷ Voelkel, E. F., Tashjian, A. H., Jr, Franklin, R., Wasserman, E. & Levine, L. *Metabolism* 24, 973–986 (1975).
- ⁸ Tashjian, A. H., Jr, Voelkel, E. F. & Levine, L. *Biochem. biophys. Res. Commun.* 74, 199–207 (1977).
- ⁹ Seyberth, H. W. *et al.* *New Engl. J. Med.* 293, 1278–1283 (1975).
- ¹⁰ Tashjian, A. H., Jr. *New Engl. J. Med.* 293, 1317–1318 (1975).
- ¹¹ Franklin, R. B. & Tashjian, A. H., Jr, *Endocrinology* 97, 240–243 (1975).
- ¹² Samuelsson, B., Granström, E., Green, K., Hamburg, M. & Hammarström, S. *A. Rev. Biochem.* 44, 669–695 (1975).
- ¹³ Corey, E. J., Nicolaou, K. C., Machida, Y., Malmsten, C. L. & Samuelsson, B. *Proc. natn. Acad. Sci. U.S.A.* 72, 3355–3358 (1975).
- ¹⁴ Robinson, D. R., Tashjian, A. H., Jr. & Levine, L. *J. clin. Invest.* 56, 1181–1188 (1975).
- ¹⁵ Seyberth, H. W., Hubbard, W. C., Morgan, J. L., Sweetman, B. J. & Oates, J. A. *Clin. Res.* 24, 370A (1976).

Inhibition of masculine differentiation in male offspring of rabbits actively immunised against testosterone before pregnancy

ACTIVE immunisation of male rabbits with testosterone-protein conjugates results in the production of antibodies capable of neutralising the biologic activity of endogenous testosterone¹. Due to the reduction of free, biologically active testosterone caused by almost total binding to antibodies the entry of this hormone into the target cells is inhibited, the metabolic clearance rate is decreased, the negative feedback to the hypothalamus is interrupted and pituitary gonadotropin release is increased. Thus, in spite of enormously elevated total plasma testosterone concentrations, atrophy of the prostate, paraprostate and seminal vesicles can be observed in suitably immunised male rabbits. In female rats actively immunised against testosterone Hillier *et al.*² also observed—despite significantly elevated total plasma testosterone concentrations—a considerable reduction of the free testosterone fraction and an elevation of FSH. Histological examination of these animals revealed development of polycystic ovaries, similar to those seen in the Stein-Leventhal syndrome in humans. These reports prompted us to study whether it is possible to fertilise female rabbits after active immunisation against testosterone, and whether in pregnancy the antibodies pass through the placenta in sufficient concentrations to neutralise the biologic activity of testosterone secreted by the foetal testes, thus selectively inhibiting the testosterone-dependent steps of male sex differentiation. We found inhibition of masculine differentiation in offspring of rabbits actively immunised against testosterone before pregnancy.

We immunised eight mature female rabbits by repeated subcutaneous injections of 2 mg testosterone-3-bovine serum albumin (T-3-BSA) dissolved in 1 ml of 0.9% saline at intervals of 2–6 weeks. For the first three injections, the antigen solution was emulsified in 1 ml of Freund's complete adjuvant. A control group of four animals was immunised against unconjugated bovine serum albumin (BSA) in an identical schedule. All animals injected with T-3-BSA developed highly specific antibodies against testosterone. Three months after beginning immunisation the titres ranged from 1:4,000 to 1:40,000 and increased after further booster injections. No antibodies to testosterone could be detected in the controls and their plasma testosterone concentrations (7–26 ng per 100 ml) were unaffected by the immunisation with BSA. In testosterone-immunised animals, however, the increase in antibody concentration was paralleled by a striking elevation of total plasma testosterone from an average of 12 ng per 100 ml before immunisation to 630–1,450 ng per 100 ml when antibody titres above 1:40,000 were reached. Regardless of these high testosterone levels, which exceed the normal range of male rabbits¹, we tried to mate these ani-

mals. After several fruitless attempts mating was successful in five of them. Altogether they gave birth to 19 males and 16 females. Throughout pregnancy the antibody titres varied from 1:15,000 to 1:40,000; the testosterone binding capacity at 0 °C ranged from 130 to 220 µg per 100 ml. The four control animals produced 11 males and 16 females. Immediately after delivery all newborns were bled and blood was collected separately. The genital tract was fixed in Bouin's mixture, embedded in paraffin, cut transversely and stained with haematoxylin eosin.

In all neonates from animals immunised with testosterone high concentrations of antibodies against testosterone were found, with specificity and association constants identical to those in the maternal plasma. The testosterone binding capacity was about 40% of that of the corresponding mother. Plasma testosterone levels were very high with mean values of 1,600 ng per 100 ml in the females and 24,000 ng per 100 ml in males. The mean testosterone levels in the female and male control neonates, in contrast, were 15 and 18 ng per 100 ml, respectively, which resembled those of normal neonatal rabbits.

In spite of their enormously elevated testosterone levels, the experimental female neonates showed normal female sex differentiation; the males, however, revealed genital malformations which can be traced back to the inactivation of testosterone by antibody binding, resulting in feminisation of internal and external genitalia. The most striking finding was the underdeveloped accessory sexual glands. In most animals the prostate and paraprostate were totally absent and the vesicular glands were reduced to rudimentary buds (Fig. 1). The bulbo-urethral glands, which in males are normally more extensively developed than in females, showed the extent of female glands only. Seminal vesicles were present in all experimental males;

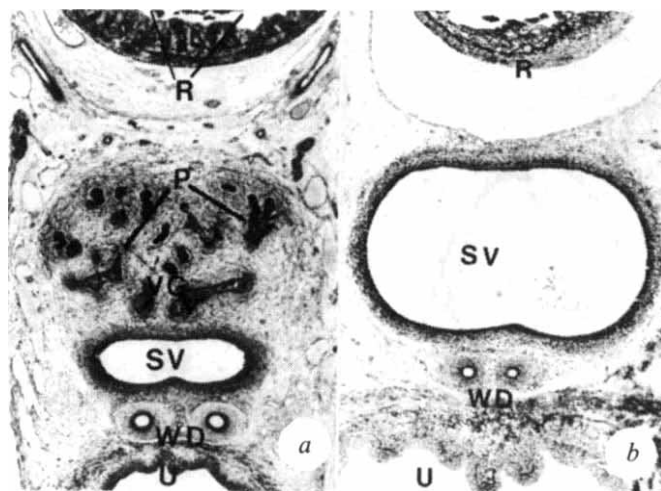


Fig. 1 Cross sections of male newborn rabbits at the level of prostate and vesicular gland. *a*, Male control (showing no difference from normal males); *b*, male immunologically deprived of testosterone. R, rectum; U, urethra; P, prostate; VG, vesicular gland; SV, seminal vesicle; WD, Wolffian duct.

however, in most of them they were less developed than in normal neonates. The Wolffian ducts were severely affected and were at the point of regression (Fig. 2). The epithelium was irregular and flat, the basal membrane had disappeared and the surrounding mesenchyme penetrated into the epithelium. Pycnosis, chromatolysis and other signs of imminent cell death predominated. The phallus of most males immunologically deprived of testosterone was also feminine (Fig. 3). The urethra corresponded to the female sinus urogenitalis rather than to the urethra of normal males. The corpus fibrosum was reduced to the

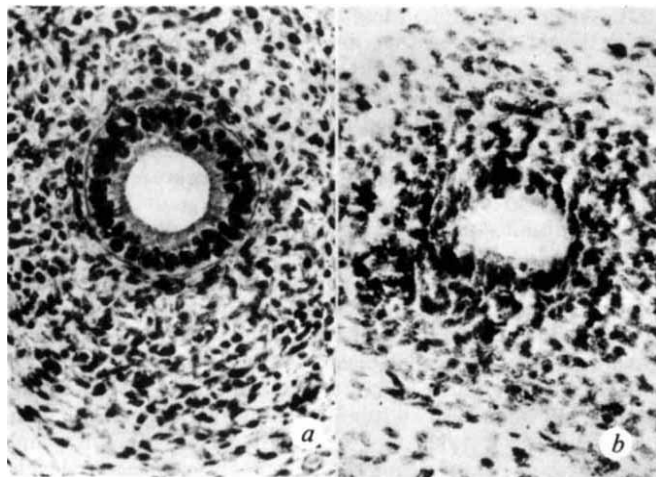


Fig. 2 Cross sections through Wolffian ducts of male newborn rabbits. *a*, Male control (showing no difference from normal males); *b*, male immunologically deprived of testosterone.

normal extent for females. As in females, the lamina glandaris was open ventrally, the urethra was located eccentrically, protruding into the perineum, and severe hypospadias could be observed. Despite these distinct manifestations of testosterone deficiency we never observed alternative development of female gonoducts. As in normal males the Müllerian ducts were completely inhibited. No abnormalities were detected in the testes, so withdrawal of testosterone as from the beginning of foetal development did not influence the gonadal differentiation. Microscopic examination of the control litters revealed normal sex differentiation in both sexes. The histological

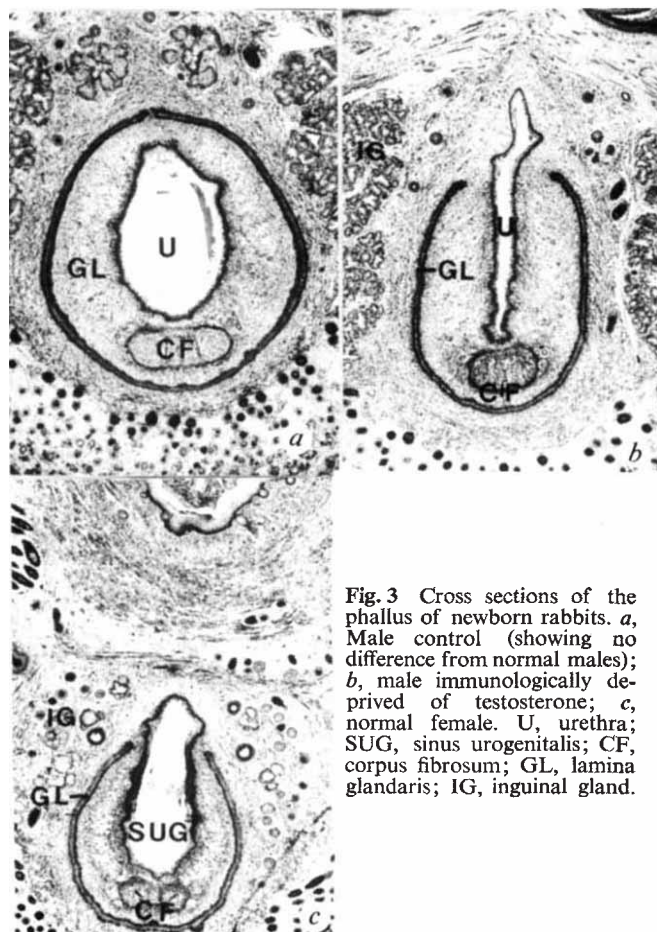


Fig. 3 Cross sections of the phallus of newborn rabbits. *a*, Male control (showing no difference from normal males); *b*, male immunologically deprived of testosterone; *c*, normal female. U, urethra; SUG, sinus urogenitalis; CF, corpus fibrosum; GL, lamina glandaris; IG, inguinal gland.

pictures were indistinguishable from those of normal female and male neonatal rabbits, respectively.

Our experiments indicate that hormones, which are essential for normal foetal development, can be neutralised in the foetuses by their respective antibodies raised in the mother, provided that the immunisation does not interfere with fertilisation. In rabbits active immunisation against testosterone does not preclude pregnancy. After passage of the placenta, the antibodies inhibit the entry of testosterone produced by the foetal testes into the cells of the developing target organs. In contrast to the experiments of Jost³, who deprived male foetuses totally of testicular activity by castration *in utero*, our experiments resulted in the selective suppression of the androgenic effects of the foetal testes, leaving that testicular factor intact which controls Müllerian duct inhibition. Thus, with respect to the foetal genital tract the effects of maternal immunisation against testosterone are comparable to those of blocking the foetal androgens by treating the pregnant mothers with cyproterone acetate, as described by Neumann *et al.*⁴ The 1,000-fold elevation of plasma testosterone in the male offspring of testosterone-immunised female rabbits suggests, however, that the antibodies exhibit additional important effects on foetal testosterone metabolism and regulation.

F. BIDLINGMAIER
D. KNORR

University of München,
Childrens' Hospital,
D-8000 München 2, FRG

F. NEUMANN

Schering AG,
D-1000 Berlin 65, FRG

Received 6 July 1976; accepted 21 February 1977.

¹ Nieschlag, E., Usadel, K. H., Wickings, E. J., Kley, H. K. & Wuttke, W. in *Immunization with Hormones in Reproduction Research* (ed. Nieschlag, E.) 155-170 (North-Holland, Amsterdam, 1975).

² Hillier, S. G., Groom, G. V., Boyns, A. R. & Cameron, E. H. D. *Nature* 250, 433-434 (1974).

³ Jost, A. *Archs Anat. microsc. Morph. exp.* 36, 271-315 (1947).

⁴ Neumann, F. *et al. Recent Prog. Horm. Res.* 26, 337-405 (1970).

Vitamin A deprivation and *Drosophila* photopigments

VITAMIN A deprivation causes a profound reduction in visual sensitivity and photopigment concentration in the eyes of both vertebrates and invertebrates^{1,2}. In vertebrates vitamin A deprivation results in degeneration of photoreceptor cells³ and decreases the number of intramembrane particles visualised in the disks of rod outer segments by freeze-fracture electron microscopy⁴. This and other evidence⁵ suggests that these particles represent rhodopsin molecules. Invertebrate photoreceptors have similar, 8-nm intramembrane particles in the stacked microvilli which form their light-sensitive rhabdomeres^{6,7}. The number of particles is comparable to the number of pigment molecules present⁸ and is dependent on the state of light adaptation⁹. We describe here a study of the effects of vitamin A deprivation on the fine structure, photopigment content, and sensitivity of *Drosophila* photoreceptors. The results support the hypothesis that most of the intramembrane particles in invertebrate rhabdomeres, like those in vertebrate photoreceptors, are rhodopsin molecules.

D. melanogaster (Canton-Special strain) were deprived of vitamin A by raising sterilised eggs on Sang's synthetic medium C (ref. 10); control flies were reared identically except that β -carotene 1.25 mg ml⁻¹ was added to the medium. White-eyed mutants (*w*) were employed in some experiments to eliminate screening pigments which interfere with spectrophotometric measurements. Fly heads were bisected, fixed for 2 h at 21 °C in half-strength fixative¹¹, and washed in cacodylate buffer. Preparations for conventional electron microscopy were postfixed in osmium tetroxide, embedded in epoxy plastic, sectioned, and stained with

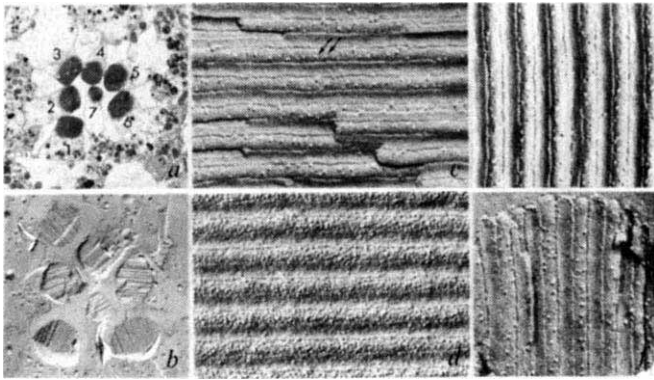


Fig. 1 Electron micrographs of *Drosophila* photoreceptors: *a*, tangential section through the central portion of an ommatidium in a vitamin A-deprived fly, showing cells R1–R6 and R7. The size and arrangement of the reticular cells and their rhabdomeres are normal ($\times 2,400$); *b*, freeze-fracture view similar to the above, demonstrating fracture faces in a vitamin A-deprived fly and the ability of the technique to demonstrate specific reticular cells ($\times 4,000$); *c*, high-magnification freeze-fracture view of rhabdomeric membranes of a peripheral photoreceptor in a vitamin A-deprived specimen—8-nm intramembrane particles (arrows) are very few in number ($\times 43,000$); *d*, freeze-fracture view of control rhabdomeric membranes, which are heavily studded with particles ($\times 43,000$); *e*, high-magnification photomicrograph of a cell R7 rhabdomere from a deprived animal, demonstrating a paucity of particles ($\times 43,000$); *f*, membrane fracture faces of a cell R8 from a deprived fly, showing a reduced intramembrane particle density ($\times 43,000$).

lead citrate. Freeze-fracture specimens were equilibrated for 8 h with 25% glycerol in *Drosophila* saline, freeze-fractured and etched for 1 min in a Balzers apparatus, and replicated by platinum and carbon shadowing. Specimens were examined with a Philips EM 301 and measurements made on prints at a magnification of $100,000\times$. Visual pigment content in homogenates prepared from exactly 100 heads each was assayed for light-induced absorbance changes in a dual-wavelength spectrophotometer¹². Visual sensitivity was measured by determining the intensity of 470-nm light needed to elicit a criterion electroretinogram (ERG) response¹² of 5 mV.

Vitamin A-deprived *Drosophila* retinas appear normal in low-power electron micrographs (Fig. 1*a, b*) and rhabdomeric dimensions fall within the normal range. The number of 8-nm intramembrane particles in rhabdomeric microvilli, however, is decreased about fourfold in comparison with controls (Fig. 1*c, d*, Table 1). Spectrophotometric measurements reveal a greater than 70-fold reduction in photopigment level (Fig. 2, Table 1). Correspondingly, visual sensitivity as measured by the ERG is reduced approximately 80-fold by vitamin A deprivation (Table 1).

The normal size and shape of the rhabdomeric microvilli in vitamin A-deprived *Drosophila* show that a full complement of rhodopsin is not required for structural integrity of the photoreceptor membrane, a finding similar to that in *Aedes*¹³ but

contrasting with that in vertebrate rods³ and moth photoreceptors¹⁴. The nature of the intramembrane particles remaining after vitamin A deprivation is unknown; because the reduction in rhodopsin measured spectrophotometrically or physiologically is much larger than the reduction in particle density, it is unlikely that the persisting particles represent rhodopsin molecules. The discrepancy of results obtained by the three techniques could be explained if some of the particles are not photopigments or if they are opsin molecules lacking chromophores. Nevertheless, the evidence indicates that the large majority of the 8-nm particles in the rhabdomeric membranes of normally reared flies are rhodopsin molecules.

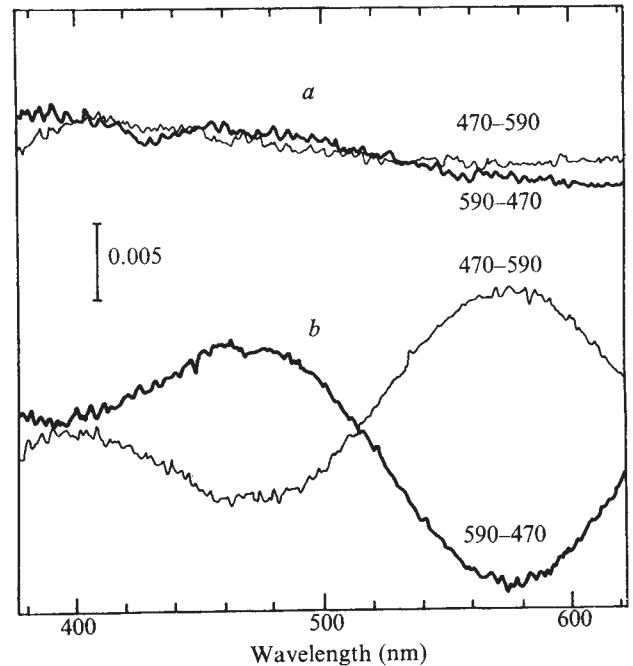


Fig. 2 Absorption spectra changes for *a*, vitamin A-deprived flies and *b*, control flies. The ordinate is the difference between the absorbances after 10 s actinic irradiations presented successively at 470 nm (2.7 mW cm^{-2}) and 590 nm (2.3 mW cm^{-2} ; thin lines) or in the opposite order (thick lines).

Each subunit or ommatidium of the *Drosophila* compound eye contains eight photoreceptors which constitute three distinct classes, each with a different photopigment¹². These three types correspond morphologically to the peripheral photoreceptors, cells R1–R6, which are blue receptors; the upper-central cell, R7, which is an ultraviolet receptor; and the lower-central cell, R8, which is a blue receptor with a different pigment from that in cells R1–R6. The preponderance of R1–R6 cell types in the retina

Table 1 Effect of vitamin A deprivation on the density of intramembrane particles, light-induced absorbance change, and electroretinogram sensitivity in *Drosophila*

	Vitamin A-deprived	Control	Deprived Control
Intramembrane particle density (particles per μm^2)*	1030 ± 31 ($n = 27$)	4214 ± 163 ($n = 6$)	0.24 ± 0.01
Light-induced absorbance change (ΔA)	<0.0002 ($n = 2$)	0.015 ± 0.002 ($n = 2$)	<0.01
Electroretinogram sensitivity ($\text{mV } \mu\text{W}^{-1} \text{ cm}^{-2}$)*	0.42 ± 0.07 ($n = 5$)	32 ± 5 ($n = 5$)	0.013 ± 0.003

Intramembrane particle densities were determined from counts of the 8-nm particles on rhabdomeric 'P' fracture faces and from areal measurements made with a Wang 600 planimetric computer. No corrections were made for the curvature of microvillar surfaces. Data from white-eyed and normally pigmented flies were not significantly different and were combined. Light-induced absorbance changes were measured with the sensitive dual-wavelength mode of the spectrophotometer at 470 and 580 nm, 10 s actinic irradiations were provided alternately at 470 nm (2.7 mW cm^{-2}) and 590 nm (2.3 mW cm^{-2}). Visual sensitivity was measured for a criterion electroretinogram response of 5 mV to 470 nm light. Data are given as means with standard errors of means; n is respectively the number of rhabdomeres scored, of pigment preparations scanned, and of flies studied.

*Difference significant at $P < 0.001$.

usually masks the contribution of R7 and R8 cells to spectrophotometric and ERG measurements, and thus prevents the determination of the chromophore in these cells. The reduction of intramembrane particles by vitamin A deprivation in all three receptor classes (Fig. 1e, f, Table 1) suggests, however, that the three spectrally distinct photopigments all use retinal (or its derivatives) as a chromophore. This result is consistent with other evidence^{1,5} that the ultraviolet-sensitive photopigments of flies are retinal-based.

This work was supported by US NIH and NSF grants to Drs S. Benzer, M. Delbrück and J.-P. Revel, whom we thank, and by grants to A. J. H. from The Alfred P. Sloan and The Merck Co. Foundations.

W. A. HARRIS
D. F. READY
E. D. LIPSON
A. J. HUDSPETH

Division of Biology,
California Institute of Technology,
Pasadena, California 91125

W. S. STARK

Department of Psychology,
Johns Hopkins University,
Baltimore, Maryland 21218

Received 2 December 1976; accepted 8 February 1977.

- ¹ Dowling, J. E. & Wald, G. *Proc. natn. Acad. Sci. U.S.A.* **44**, 648–661 (1958).
- ² Goldsmith, T. H., Barker, R. J. & Cohen, C. F. *Science* **146**, 65–67 (1964).
- ³ Dowling, J. E. & Wald, G. *Proc. natn. Acad. Sci. U.S.A.* **46**, 587–608 (1960).
- ⁴ Jan, L.-Y. & Revel, J.-P. *J. Cell Biol.* **62**, 257–273 (1974).
- ⁵ Hong, K. & Hubbell, W. L. *Proc. natn. Acad. Sci. U.S.A.* **69**, 2617–2621 (1972).
- ⁶ Eakin, R. M. & Brandenburger, J. L. *Am. Zool.* **15**, 851–863 (1975).
- ⁷ Eakin, R. M. & Waterman, T. H. *Cell Tiss. Res.* **169**, 419–434 (1976).
- ⁸ Fernandez, H. R. & Nickel, E. E. *J. Cell Biol.* **69**, 721–732 (1976).
- ⁹ Brandenburger, J. L., Reed, C. T. & Eakin, R. M. *Am. Zool.* **15**, 782 (1975).
- ¹⁰ Sang, J. H. *J. exp. Biol.* **33**, 45–72 (1956).
- ¹¹ Karnovsky, M. J. *J. Cell Biol.* **27**, 137A–138A (1965).
- ¹² Harris, W. A., Stark, W. S. & Walker, J. A. *J. Physiol.* **256**, 415–439 (1976).
- ¹³ Brammer, J. D. & White, R. H. *Science* **163**, 821–823 (1969).
- ¹⁴ Carlson, S. D., Gemme, G. & Robbins, W. E. *Experientia* **25**, 175–177 (1969).
- ¹⁵ Paulsen, R. & Schwemer, J. *Biochim. biophys. Acta* **283**, 520–529 (1972).

Storage of cannabinoids by *Arctia caja* and *Zonocerus elegans* fed on chemically distinct strains of *Cannabis sativa*

THE larvae of the warningly coloured tiger moth *Arctia caja* (L.) (Lepidoptera) and the grasshopper *Zonocerus elegans* (Thunberg) (Acridoidea) are polyphagous feeders with a predilection for poisonous food plants. These species can sequester and store various secondary plant substances such as cardiac glycosides and pyrrolizidine alkaloids^{1,2}, which presumably function as predator deterrents. We report here that cannabinoids ingested with the leaves of *Cannabis sativa* L. are also stored by both insects.

There is good evidence that *C. sativa* contains at least two chemical races or strains, one rich in the psychoactive substance Δ -1-tetrahydrocannabinol (THC) and the other in the inactive cannabidiol (CBD)³, and accordingly one of each type was used in our work.

A. caja larvae, derived from a single egg batch from a wild female caught at Ashton Wold, were allowed to feed freely either on a Mexican THC-rich strain (referred to as THC strain) or a Turkish CBD-rich strain (referred to as CBD strain), with no alternative food offered. Leaves from matched shoots of both strains were taken for analysis as controls. Feeding continued for 44 d at 24 °C and 50% relative humidity. Another batch of larvae was reared to the third instar on the CBD strain, then transferred to the THC strain. Controls were fed on *Senecio jacobaea* L. Only nine wild caught *Zonocerus elegans* nymphs (from Nigeria, where they were feeding on cassava) were available. Because we found the Mexican strain of *Cannabis* had proved lethal for hoppers of *Schistocerca gregaria* (Forsk.) we allowed *Z. elegans* to feed on both strains indiscriminately. They showed no preference but fed

sparingly. One specimen died and the rest were killed after 6 week continuous feeding on *Cannabis*. For the last 3 d before analysis the diet was changed to grass to empty the gut of cannabinoids. Eight whole insects, four exuviae and a sample of frass were analysed.

The *A. caja* larvae fed on THC strain were stunted (Fig. 1) and did not survive beyond the third instar. Those fed on the CBD strain developed more slowly than the controls, but pupated successfully after 7–8 weeks of larval life.

About 30% of larvae reared to the third instar on CBD strain and then transferred to the THC strain developed and pupated successfully. Subsequently, further attempts were made to rear larvae on the THC strain, and on one occasion four specimens reached the fifth instar after 9 months' continuous feeding, and, of these, one pupated and the female which emerged mated and laid fertile eggs.

The vegetative leaves of the Mexican strain contained 19.3 mg of THC per g dry weight, while those of the Turkish strain contained 10.2 mg of CBD per g dry weight. Thus the greater toxicity of the Mexican strain may have been due to greater toxicity of THC or simply to a greater absolute amount of cannabinoid: other differences between the two types may contribute to the more lethal effect of the Mexican THC strain.

The larvae (starved for 24 h before analysis), their frass, exuviae and pupae were examined for cannabinoids. Insect samples were extracted for 18 h with ethanol at room temperature and the extracts were assayed as described before⁴. Quantitative identification of cannabinoids was checked by thin-layer chromatography⁵. The residue was heated under reflux with 0.1 N NaCl for 1 h and then

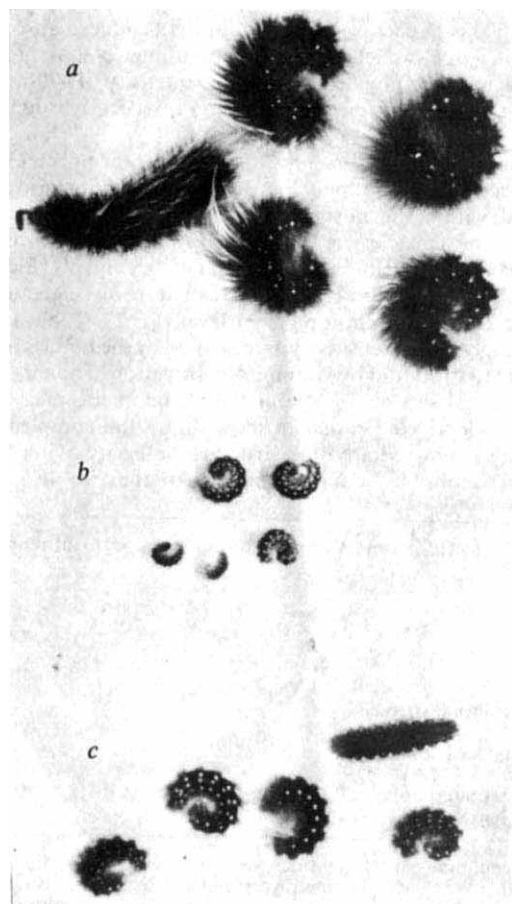


Fig. 1 Larvae reared for a, 32 d on ragwort (*Senecio jacobaea*); b, 32 d on the THC-rich strain of *Cannabis*; and c, 32 d on the CBD-rich strain of *Cannabis*.

Table 1 Cannabinoid contents (mg per g fresh weight) of *Arctia caja* fed on *Cannabis sativa**

	CBD type throughout larval growth	CBD type to 3rd instar, and then THC type	Feeding regime THC type to death (3rd instar)
Larvae			
CBD	trace	0.21 (0.01)†	NE
THC	0	0.99 (0.07)	NE
Pupae			
CBD	0	0	NE
THC	0	0	NE
Exuviae	(mg per g dry weight)		
CBD	0.1 (0.0012)	0.28 (0.004)	0
THC	0	2.6 (0.04)	1.4 (0.003)
Frass	(mg per g dry weight)		
CBD	2.1	NE	0
THC	0	NE	3.0

*The coefficient of variation for a single assay involving all stages is only 2.7% (ref. 4). Where sufficient material was available it was analysed in duplicate.

†Figures in parentheses indicate the weight of cannabinoid in mg in each insect.

NE, not examined.

extracted with diethyl ether. This extract was also examined as above.

Table 1 shows that specimens reared throughout larval growth on CBD strain contained only traces of CBD, insufficient for quantitative analysis. Acid hydrolysis of the residue released a trace of a compound which gave a positive colour reaction for cannabinoids but did not correspond to any available standards on gas chromatography. Such traces were also found in the pupae and may represent a metabolite of CBD, present as a glucoside. CBD, as the acid, in which form it appears in the plant, was present in the exuviae (cast skins) at a concentration of 0.0012 mg per exuvia. On the other hand, larvae reared to the third instar on the CBD strain then transferred to the THC strain, stored more CBD per insect (0.01 mg) in the fifth instar than those fed solely on the CBD strain, and in addition each contained 0.07 mg of THC. This was also present mainly as the acid, but some decarboxylated THC was identified.

The exuviae of insects reared exclusively on the THC strain until death contained relatively high concentrations of THC acid. Thus, measured as mg of cannabinoid per g of insect material, the exuviae of insects reared on the THC strain contained more than ten times as much cannabinoid as those feeding on the CBD strain. Those reared to the third instar on the CBD strain and then switched to the THC strain contained 25 times as much.

In both sets of larvae at least 80% of the cannabinoids recovered was found in the relatively small sample of frass analysed (Table 1). This shows that they excrete much more cannabinoid than they store; however the concentration in the frass was much less than that in the plant material, which was the sole article of diet, so that most of the cannabinoid eaten must have been metabolised.

Most of the cannabinoid recovered from *Z. elegans* (Table 2) occurred in the frass. The weight of THC in the whole insect was remarkably similar to that in full grown *A. caja* larvae, but a much smaller proportion was stored in the integument. CBD was lacking in both the insects and their exuviae, although it occurred in the frass, thus indicating that *Z. elegans* can metabolise this compound efficiently.

The most striking aspect of the storage of THC by *A. caja* is the relatively large amounts concentrated in the cuticle. Thus the insect can rid itself of much of the toxic material not voided in the frass, but accumulates it between moults at a site where it could function as a 'predator deterrent'. Mice, which are voracious feeders on larval Lepidoptera, show a reduction of spontaneous activity when given an oral dose of 0.013–0.025 mg of pure THC (J. Pickens, unpublished). Thus, one larva containing 0.07 mg THC would be above the threshold

dose for pharmacological activity. Although the accumulation of toxins in the cuticle has not been noted before, it may be a widespread method for the storage of deterrent secondary plant substances, especially in aposematic larvae which are followed by a cryptic adult stage⁶.

When we offered newly hatched *A. caja* larvae (20 trials) a choice of leaves from both strains of the plant (matched for size, age and position on the stem) they showed a definite preference for the Mexican strain. This may be due to factors unconnected with the type and concentration of cannabinoids, but should these compounds exert a fatal fascination for tiger caterpillars it suggests another subtle system of insect control by plants.

Table 2 Cannabinoid contents of *Zonocerus elegans* fed on *C. sativa*

	THC content*	CBD content*
Whole insect (mg per insect)	0.06	0
Exuviae (mg per g dry weight)	0.006	0
Frass (mg per g dry weight)	2.95	0.97

*No additional cannabinoid was released by acid hydrolysis.

Our results demonstrate the versatility of moth and grasshopper in dealing with poisonous plant substances encountered during feeding. Both are preadapted to the storage of cannabinoids, for *C. sativa* has not been recorded as a food plant in nature. Because of widespread cultivation of *C. sativa* the ranges of the plant and *A. caja* now overlap. Furthermore, both store the more toxic THC rather than the relatively innocuous CBD. This is reminiscent of the aposematic butterfly *Danaus plexippus* (L.) and grasshopper *Poekilocerus bufonius* (Klug) which also store the more active cardiac glycosides⁷ ingested with their food plants.

We thank Dr Elizabeth Bernays for the gift of living *Zonocerus*.

MIRIAM ROTHSCHILD

Ashton, Peterborough

M. G. ROWAN

J. W. FAIRBAIRN

School of Pharmacy,
Brunswick Square,
London WC1, UK

Received 30 December 1976; accepted 16 February 1977.

- 1 Rothschild, M. in *Insect/Plant Relationships. Symp. R. ent. Soc. Lond.* 6 (ed. van Emden, H. F.), 59–83 (Blackwells, Oxford, 1972).
- 2 Bernays, E., Edgar, J. A. & Rothschild, M. *J. Zool. Lond.* (in the press).
- 3 Fairbairn, J. W. & Liebmann, J. A. *J. pharm. Pharmac.* 26, 413–419 (1974).
- 4 Fairbairn, J. W. & Liebmann, J. A. *J. pharm. Pharmac.* 25, 150–155 (1973).
- 5 Maunders, M. J. de Faubert *J. pharm. Pharmac.* 21, 334–335 (1969).
- 6 Marsh, N. & Rothschild, M. *J. Zool. Lond.* 174, 89–122 (1974).
- 7 Reichstein, T. *Naturwiss. Rdsch.* 29, 499–511 (1967).

Nature of recombinants produced by the RecBC and the RecF pathways in *Escherichia coli*

BASED ON analysis of various recombination deficient mutants, at least three different pathways of chromosomal recombination have been suggested in *E. coli* K12: the RecBC, the RecF and the RecE pathways¹⁻³. Our present knowledge of initial and final DNA structures and of the intervening events is very limited. Only some of the genes controlling each pathway have been identified^{3,4}; the products of a few of these have been isolated and characterised⁵⁻¹⁰; little is known about the specific steps at which these products participate in the respective pathways. The role of exonuclease V, the product of the *recB* and *recC* genes, has been studied¹¹⁻¹⁵. Recipients deficient in this enzyme give greatly reduced yields of viable recombinants¹¹, but support certain types of recombinational events with relatively high efficiency^{12,13,15}. ExoV has thus been considered to be more important in determining viability of the recombinant cells than in the recombinational process itself. It has been suggested, on the basis of transformation and transduction experiments, that the recombinant products of the RecBC and the RecF pathways may be structurally different^{16,17}. We present here results from conjugation crosses of *E. coli* K12 which demonstrate that the viable recombinants produced by the RecBC and the RecF pathways are significantly different in terms of the density of genetic exchanges present in them. A preliminary account of this work has already been presented¹⁸.

A simple mathematical formulation of *E. coli* conjugation system¹⁹ shows that the recombinational process in this system can be visualised as consisting of two operationally separable parts: (1) the initiation of the recombinational process at randomly distributed points on the chromosome, and (2) the development around these points of short regions of recombination (RRs), each of which may contain several genetic exchanges. Increase in the probability of initiation of the RRs is reflected in increased values of two-point recombination frequencies (R1) between pairs of markers, while increase in the mean number of exchanges per RR, is reflected in increased values of R2(1), which is a measure of the frequency of additional exchanges close to a selected exchange (Fig. 1). A comparison of R1 and R2(1) values in recombinants produced by the different recombinational pathways can thus give information about the differences present in the final products of these pathways and about the earlier events.

MD1763, a *pyrA* derivative of HfrH was mated with an *ara*, *thr*, *str-r*, F⁻ recipient, AB1157 and its *recB recC*, *recB recC sbcB*, and *recF* derivatives (Table 1). Selection was made for *ara*⁺ *str-r*, *ara*⁺ *pyrA*⁺ *str-r* and *ara*⁺ *pyrA*⁺ *thr*⁺ *str-r* recombinant types. The values of R1 and R2(1) calculated from these (Table 2) are strongly dependent on the recombinational pathways functioning in the recipients. It seems that the recombinational process controlled by the RecF pathway involves a higher



Fig. 1 Configuration of a typical three-point cross; R1 is the two-point recombination frequency in region 1 and is the number of *a*⁺*b*⁺*str-r* recombinants per *a*⁺*str-r* recombinant. R2(1) is the frequency of recombination in region 2 in the subpopulation selected for recombination in region 1 and is given by the number of *a*⁺*b*⁺*c*⁺*str-r* recombinants per *a*⁺*b*⁺*str-r* recombinant.

density of exchange clusters along the chromosomal length and a higher number of recombinational exchanges per cluster as compared to the RecBC pathway.

The markers involved in the above intergenic cross are comparatively widely spaced, each of the two intervals (1 and 2) measuring a little more than half a minute on the standard genetic map of *E. coli* K12 (ref. 20). To see whether similar pathway-dependent effects are present in recombination involving closely spaced markers, two-point crosses involving markers, (1) within a single gene (*araC*), and (2) in two neighbouring genes (*araB* and *araC*) of a single operon, were performed for all the above-mentioned recipient backgrounds. R1 values were determined in each case (Table 3), and the effect of mutations in the RecBC or the RecF pathway on R1 values for closely spaced markers is essentially similar to that for widely spaced markers (Table 2).

Table 2 Conjugational crosses between MD1763 donor strain and different recipient strains

Recipient strains	R1	R2(1)
AB1157	0.062 ± 0.017	0.16 ± 0.04
AB2470C	0.24 ± 0.04	0.12 ± 0.04
JC7623	0.24 ± 0.05	0.25 ± 0.05
JC9239	0.055 ± 0.015	0.11 ± 0.03

Conjugation experiments were performed as follows: exponentially growing cultures (2–4 × 10⁸ cells per ml) of donor and recipient cells were mixed in the ratio 1:5 and incubated for 5 min, then the mating mixtures were diluted 1:50 in liquid BT 8 nutrient broth containing tryptone and allowed to mate for another 55 min with gentle shaking. The mating mixtures were then diluted 1:1 in BT containing 200 µg per ml⁻¹ streptomycin, blended and incubated with shaking for 4 h, then plated on prewarmed selection plates¹⁴, spread with 0.7% topping agar and incubated for 40 h before counting the number of recombinant colonies. All operations were performed at 37°C. The results are averages of at least three experiments. The above cross is similar to the typical three-point cross shown in Fig. 1, with *ara-14*, *pyrA53* and *thr-1* representing markers *a*, *b* and *c*, respectively. Both R1 and R2(1) values are highest in JC7623, which is expected to produce most of its viable recombinants via the RecF pathway. The lowest values are obtained in JC9239, which lacks the RecF pathway and is mostly dependent on the RecBC pathway for recombinant production. The wild type AB1157 gives intermediate values for these frequencies, while AB2470C, which lacks *exoV* gives an R1 value comparable to JC7623 and an R2(1) value, somewhat lower than the wild type.

Table 1 *E. coli* K12 strains used in this study

Strains	<i>recB</i>	Rec genotype <i>recC</i>	<i>recF</i>	<i>sbcB</i>	Other markers	Sex	Source or ref.
AB1157	+	+	+	+	<i>thi-1</i> , <i>thr-1</i> , <i>leu-6</i> , <i>lacY1</i> , <i>mtl-1</i> , <i>xyl-5</i> , <i>ara-14</i> , <i>galK2</i> , <i>his-4</i> , <i>proA2</i> , <i>argE3</i> , <i>tsx-33</i> , <i>sup-37</i> (amber), <i>str-31</i>	F ⁻	A. J. Clark
AB2470C	21	22	+	+	Same as AB1157	F ⁻	J. Tomizawa
JC7623	21	22	+	15	Same as AB1157	F ⁻	A. J. Clark
JC9239	+	+	143	+	Same as AB1157	F ⁻	A. J. Clark
MD1763	+	+	+	+	<i>pyrA53</i> , <i>thi</i>	HfrH	19
MD2116	+	+	+	+	<i>araB113</i> , <i>trpR1</i> , <i>dra-2</i>	HfrH	This study
MD2121	+	+	+	+	<i>araC191</i> , <i>trpR1</i> , <i>dra-2</i>	HfrH	This study

MD2116 is an *araB* and MD2121 is an *araC* derivative of WP1767 (ref. 19); *araB* and *araC* mutations were isolated after mutagenesis with 2-aminopurine.

The comparable (three to fourfold) increase in R1 in AB2470C and JC7623 indicates that in the absence of *exoV*, the probability of initiating RR in a given chromosomal length increases. As most current models of recombination^{21,22} postulate the presence of single-stranded breaks (nicks) in DNA as an essential prerequisite for initiating the recombinational process, it is reasonable to infer that, in the absence of *exoV*, the density of such nicks is higher. This is consistent with the suggestion²³ that *recB*⁺ *recC*⁺ gene products are involved in the repair of chain breaks that arise during cell growth. The alternative possibility that *exoV* has no effect on the mean density or distribution of RR and that the increase in R1 values is simply a reflection of higher selective survival (for some unknown reason) of the recombinant subpopulation with higher number of RR seems rather improbable: it would be difficult to understand why AB2470C and JC7623, which give widely different yields of viable recombinants, should give comparable increases in R1 values.

Table 3 Two-point recombination frequencies (R1) between pair of *ara* markers in different recipient strains

Recipient strains	Donor strains	
(<i>ara-14</i>)	MD2116 (<i>araB113</i>)	MD2121 (<i>araC191</i>)
AB1157	0.0095 ± 0.0012	0.00047 ± 0.00010
AB2470C	0.0253 ± 0.0080	0.00138 ± 0.00020
JC7623	0.0278 ± 0.0060	0.00161 ± 0.00003
JC9239	0.0090 ± 0.0026	0.00031 ± 0.00005

The experimental procedures were similar to those in Table 2. The order of *ara* mutations was *pyrA53 araB113 araC191 ara-14 leu*. To calculate the frequency of recombination between different *ara* markers, the nearby *leu* was used as a reference marker. The frequencies given represent ratios of *ara*⁺ *str-r* recombinants to *leu*⁺ *str-r* recombinants produced in the respective crosses and are averages of at least three independent experiments in each case.

The considerably higher value of R2(1) in JC7623 than in AB2470C indicates that the deficiency of exonuclease I increases the mean number of exchanges per RR which may be due either to an increased exchange density within these regions of recombination or to an increase in the mean length of these regions or both. The second alternative more easily explains the improved recovery of recombinants in the *exoI* deficient *recB recC* cells, and is consistent with results in P1-mediated transduction crosses²⁴. These observations support a model (manuscript in preparation) which postulates that the RecBC pathway preferentially promotes integration of double-stranded donor DNA segments which may have short single-stranded ends, whereas the RecF pathway mostly promotes integration of single-stranded donor segments.

We thank A. J. Clark and J. Tomizawa for supplying *E. coli* strains.

S. K. MAHAJAN
A. R. DATTA

Biology and Agriculture Division,
Bhabha Atomic Research Centre,
Trombay, Bombay 400085,
India

Received 6 October 1976; accepted 18 February 1977.

- Clark, A. J. *A. Rev. Microbiol.* **25**, 438–464 (1971).
- Clark, A. J. *A. Rev. Genet.* **7**, 67–86 (1973).
- Gillen, J. R. & Clark, A. J. in *Mechanisms in Recombination* (ed. Grell, R. F.) 123–136 (Plenum, New York, 1974).
- Horii, Z. I. & Clark, A. J. *J. molec. Biol.* **80**, 327–344 (1973).
- Goldmark, P. J. & Linn, S. *Proc. natn. Acad. Sci. U.S.A.* **67**, 434–441 (1970).
- Goldmark, P. J. & Linn, S. *J. biol. Chem.* **247**, 1849–1860 (1972).
- Tanner, D., Nobrega, F. G. & Oishi, M. *J. molec. Biol.* **67**, 513–516 (1972).
- Lieberman, R. P. & Oishi, M. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4816–4820 (1974).
- Kushner, S. R., Nagaishi, H., Templin, A. & Clark, A. J. *Proc. natn. Acad. Sci. U.S.A.* **68**, 824–827 (1971).
- Kushner, S. R., Nagaishi, H. & Clark, A. J. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3593–3597 (1974).
- Low, B. *Proc. natn. Acad. Sci. U.S.A.* **60**, 160–167 (1968).
- Wilkins, B. M. *J. Bact.* **98**, 599–604 (1969).
- Hall, J. D. & Howard-Flanders, P. *J. Bact.* **110**, 578–584 (1972).
- DeHaan, P. G., Hoekstra, W. P. M. & Verhoeff, C. *Mutat. Res.* **14**, 375–380 (1972).
- Birge, E. A. & Low, K. B. *J. molec. Biol.* **83**, 447–457 (1974).
- Wackernagel, W. & Radding, C. in *Mechanisms in Recombination* (ed. Grell, R. F.) 111–121 (Plenum, New York, 1974).

- Basu, S. K. & Oishi, M. *Nature* **253**, 138–140 (1975).
- Datta, A. R. & Mahajan, S. K. *Fifteenth Annual Conference of Association of Microbiologists of India, Bangalore*, 19–21 December (1974).
- Mahajan, S. K. thesis, Univ. Pennsylvania (1971).
- Bachmann, B. J., Low, K. B. & Taylor, A. L. *Bact. Rev.* **40**, 116–167 (1976).
- Hotchkiss, R. D. *A. Rev. Microbiol.* **28**, 445–468 (1974).
- Messelson, M. S. & Radding, C. M. *Proc. natn. Acad. Sci. U.S.A.* **72**, 358–361 (1975).
- Capaldo, F. N. & Barbour, S. D. *J. molec. Biol.* **91**, 53–66 (1975).
- Crawford, I. P. & Preiss, J. J. *J. molec. Biol.* **71**, 717–733 (1972).

High-LET radiations induce a large proportion of non-rejoining DNA breaks

THE mechanisms by which mammalian cells are killed by ionising radiation have not been explained at the molecular level and radiations with a high linear energy transfer (LET) can provide an important tool for investigating these mechanisms. High-LET radiations, such as neutrons, π -mesons and low-energy heavy ions are known to kill bacteria^{1,2}, yeast^{3,4}, and mammalian cells *in vitro*^{5–9} more efficiently per unit dose than radiations with diffuse patterns of ionization, or low-LETs, such as γ or X rays. Other radiobiological phenomena associated with high-LET radiations are a reduced effect of chemical modifiers, for example, oxygen¹⁰, on cellular radiation sensitivity and reduction or loss of cellular ability to recover from radiation damage between split radiation doses^{5,8}. Because of these and other attributes, including the favourable depth-dose distribution of heavy ions and π -mesons, high-LET radiations are being actively considered for use in cancer radiation therapy^{11,12}, and a thorough understanding of their biological effects is necessary for them to be used to advantage clinically. We report here that, over an LET range of 1–1953 keV μm^{-1} , there is an excellent correlation between the efficiency of exponential (single-hit) cell killing and the induction of non-rejoining DNA strand breaks, as measured on alkaline sucrose gradients. This correlation implies that non-rejoined breaks are a cause of cell death.

We measured DNA strand break induction and rejoining in Chinese hamster V79 S171 cells¹³. X rays (300 kVp, 1.6 mm Cu h.v.l.) and low-energy heavy ions from the Lawrence Berkeley Laboratory 88-inch cyclotron and heavy-ion linear accelerator provided radiations with the various LETs used (Table 1).

Table 1 Heavy ion parameters

Ion	Energy (MeV a.m.u. ⁻¹)	LET* (keV μm^{-1})
Proton	4.0	9.4
Proton	1.15	27
⁹ Be	4.0	151
¹² C	3.8	332
²⁰ Ne	3.8	827
⁴⁰ Ar†	3.9	1953
⁴⁰ Ar	1.5	2900

*LET in water.

†Three experiments, with ion energies of 4.6, 4.2 and 3.0 MeV a.m.u.⁻¹ are grouped here.

As LET increased from 1 keV μm^{-1} with X rays to 1953 keV μm^{-1} with ⁴⁰Ar ions, DNA strand break induction decreased from $2.67 \pm 0.14 \times 10^{-4}$ to $0.48 \pm 0.03 \times 10^{-4}$ breaks per 10^8 daltons per rad (Fig. 1a). The percentage of these breaks that did not rejoin in post-irradiation incubation of 8½ h or more, however, increased with LET from $1.4 \pm 0.4\%$ for X rays to $17.9 \pm 1.5\%$ at 1953 keV μm^{-1} (Fig. 1b).

The data of Fig. 1a and b were combined to obtain the efficiency for the induction of non-rejoining breaks, normalised to the value obtained for X rays. This yielded a relative efficiency for non-rejoining break induction as a function of LET (Fig. 2). The peaked response obtained is similar to that found for killing of some strains of bacteria^{1,2} and yeast^{3,4}, for induction of mutations in yeast¹⁴, for killing of mammalian cells *in vitro*^{5–7}, and for induction of chromosome aberrations in mammalian cells *in vitro*⁷.

Killing of mammalian cells by ionising radiation can be described in terms of two classes of radiation interactions¹⁵⁻¹⁷: an irreversible lethal class, which follows single-hit kinetics and leads to an initially non-zero, negative slope on log survival against dose curves; and a sub-lethal class, which may or may not be reversible and which leads to a sigmoidal survival curve. Most of

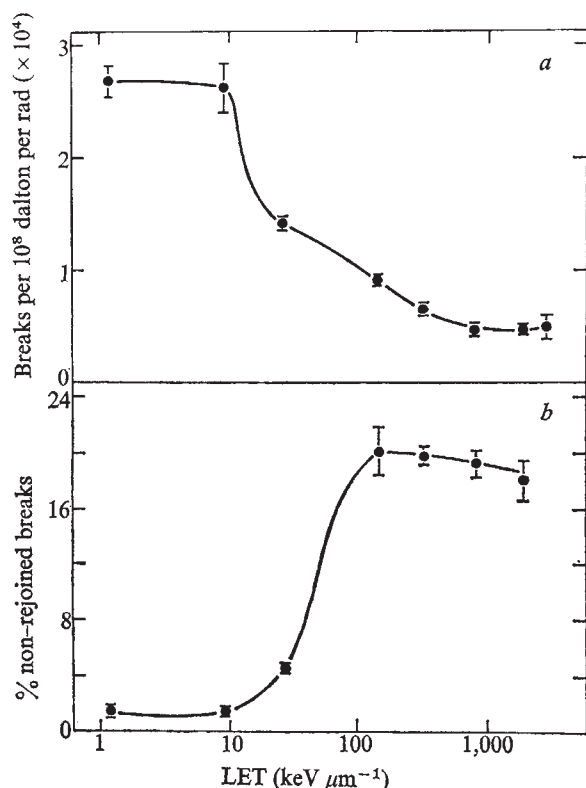


Fig. 1 Induction of DNA strand breaks by X ray and heavy ion irradiation. V79 S171 cells were grown as monolayers on glass dishes and labelled with ^{14}C -thymidine ($0.07 \mu\text{Ci ml}^{-1}$, 57 Ci mol^{-1}) for 12–14 h. They were then exposed to X rays or to the heavy ion radiations indicated in Table I, and allowed to grow for 0–12 h before collecting for gradient analysis. To eliminate the need for alkaline sucrose gradient analysis immediately, cells were frozen and gradient analysis was performed up to 1 month later. For collecting, cells were rinsed twice with citrate saline (0.75 M sodium citrate, 0.075 M sodium chloride) and 0.8 ml of citrate saline was added to cover the monolayer. The entire dish was then pressed against a methanol-coated thin aluminium plate resting on powdered dry ice. This gave very rapid freezing (within 2–3 s) of the cell monolayer and the citrate-saline solution that covered it. Dishes were placed in deep freeze (-76°C) for storage. When gradient analysis was to be performed, cell dishes were thawed in 3–4 min at room temperature. Cells were detached from the surface by gentle repipetting of the citrate-saline solution covering them, and then an equal volume of lysis solution, consisting of 0.5 M NaOH, 0.02 M $\text{Na}_2\text{-EDTA}$ and 0.1% NP-40 (non-ionic detergent) was added. Lysis was allowed to proceed for $1\frac{1}{2}$ –2 h, and 1.0 ml of the lysate was transferred to the top of an alkaline sucrose gradient. Transfer was performed with a wide-bore pipette tip connected by a Tygon tube to a motor-driven syringe. Alkaline sucrose gradients (5–20%) were formed from 5% sucrose containing 0.1 M NaOH, 0.9 M NaCl and 0.01 M $\text{Na}_2\text{-EDTA}$ (pH 12.15) and 20% sucrose containing 0.15 M NaOH, 0.85 M NaCl and 0.1 M $\text{Na}_2\text{-EDTA}$ (pH 11.20). Thirty min after the cell lysate was transferred, gradients were centrifuged at 19,700 r.p.m. in an SW 27 rotor for times that brought the peak of the DNA profiles approximately 75% of the distance down the tube. Thirty fractions per gradient were collected by puncturing the tube bottom, the radioactivity per fraction was counted, and molecular weights (weight average and number average) and the numbers of DNA breaks were calculated as before^{27,28}, using phage T4 DNA as a molecular weight standard. A number average molecular weight of approximately 2×10^8 was calculated for DNA from unirradiated V79 cells. *a*, Efficiency of induction of strand breaks. Cells were irradiated at 4°C with doses between 3 and 45 krad and were analysed for strand breaks without subsequent incubation at 37°C . *b*, Percentage of non-rejoining breaks, that is, DNA breaks in (*a*) that did not rejoin in $8\frac{1}{2}$ h or more of post-irradiation incubation at 37°C . These percentages were independent of dose (data not shown). Rejoining data were not obtained for the $2,900 \text{ keV } \mu\text{m}^{-1}$ ^{40}Ar ion irradiations.

the high-LET, mammalian cell studies cited above demonstrate that the single-hit component of cell killing predominates for high-LET radiations, yielding linear survival curves on log plots.

Todd^{18,19} analysed extensive high-LET survival data for T-1 human kidney cells in terms of such single-hit and sigmoidal components. Relative efficiencies for single-hit killing, equivalent to relative efficiencies measured at high survival levels, can be calculated from Todd's data. These are in excellent agreement with the relative efficiencies for non-rejoining DNA break induction found here (Fig. 2). These non-rejoining break data are also in close agreement with other high-LET data for single-hit killing of Chang human liver cells¹⁹ and Chinese hamster CH2B2 cells⁷ (not shown). The implication is that non-rejoining DNA breaks cause single-hit, irreversible cell death. If this is true, then non-rejoining breaks would constitute the predominant lethal lesion for high-LET radiations. At low-LETs, their contribution to cell killing would depend upon such factors as dose and dose rate, becoming relatively more important, compared to sublethal damage, as dose or dose rate was reduced.

There is evidence to support the contention that non-rejoining DNA breaks might result in cell death. Some *Escherichia coli* mutants that cannot rejoin X-ray-induced DNA breaks exhibit extreme X-ray sensitivity²⁰. Furthermore, when ^{125}I is incorporated into mammalian DNA, its decay induces a high percentage of DNA breaks that are not rejoined²¹, whereas breaks induced by ^3H or X rays are rejoined^{21,22}. ^{125}I has been shown to be more lethal per decay than ^3H and more lethal per rad than X rays²³. Also, the size of daughter DNA synthesised in cells irradiated with either high- or low-LET radiations does not exceed the size of the parental DNA in up to 12 h of post-irradiation synthesis (our work, in preparation). This indicates that non-rejoining breaks in the parental DNA serve as permanent barriers to synthesis of daughter DNA and again suggests that non-rejoining DNA breaks are lethal lesions.

The alkaline sucrose gradient technique used here detects three classes of DNA damage as breaks: single-strand breaks, double-strand breaks, and alkali-labile lesions. Thus, it is not possible to determine the exact nature of the non-rejoining breaks detected in this study. But, a peaked response with LET for the induction of

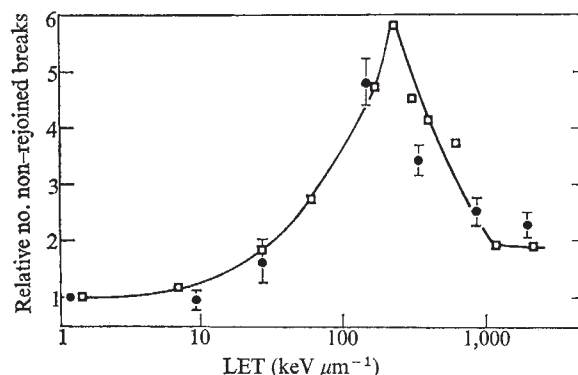


Fig. 2 ●, Relative efficiencies for non-rejoining break induction at various LETs: values obtained by combining results in Fig. 1a and b and normalising to the value obtained for X rays. □, Relative efficiencies for single-hit cell killing: these values were derived from data of Todd^{18,19} for T-1 human kidney cells.

any test effect, such as we found for non-rejoining breaks, implies that two or more radiation-target interactions are required to induce the effect²⁴. This type of induction kinetics is consistent with the induction of DNA double-strand breaks. There is also direct evidence that high-LET radiations induce double-strand breaks more efficiently in phage²⁵ and mammalian²⁶ DNA than do low-LET radiations. In addition, there is experimental evidence that non-rejoining DNA breaks induced by γ rays are actually double-strand breaks²⁹. Thus, it seems likely that DNA double-strand breaks comprise at least part of the non-rejoining breaks seen here in alkali after high-LET radiations. Other possibilities should not be excluded, however. For example, it might not be

possible for intrastrand DNA breaks within a certain critical distance of each other to be repaired, or a break near some other lesion, such as a protein-DNA cross-link, might be inaccessible to repair enzymes. As is the case with double-strand breaks, high-LET radiations should induce these hypothetically non-repairable double lesions more efficiently than low-LET radiations.

Cell killing by ionising radiation can be characterised by two independent mechanisms having lethal, single-hit and sublethal, multi-hit attributes. Our results offer a molecular explanation of the lethal, single-hit component, relating it to non-rejoining DNA strand breaks. The identity of other lesions responsible for the sublethal component remains undefined, although it has been postulated that these lesions are rejoinable DNA single-strand breaks^{17,29}.

We thank Drs Robert B. Painter and Cameron J. Koch for helpful discussions, and Dr Graeme P. Welch for design and operation of accelerator-related equipment. This work was supported by the US ERDA.

MARK A. RITTER *
JAMES E. CLEAVER

Laboratory of Radiobiology,
University of California,
San Francisco, California 94143

CORNELIUS A. TOBIAS

Donner Laboratory,
University of California
Berkeley, California 94720

Received 13 December 1976; accepted 8 February 1977.

*Present address: Department of Physiology, Harvard University, School of Public Health, Boston, Massachusetts 02115.

- ¹ Munson, R. J., Neary, G. J., Bridges, B. A. & Preston, R. J. *Int. J. radiat. Biol.* **13**, 205–224 (1967).
- ² Powers, E. L., Lyman, J. T. & Tobias, C. A. *Int. J. radiat. Biol.* **14**, 313–330 (1968).
- ³ Sayeg, J. A., Birge, A. C., Beam, C. A. & Tobias, C. A. *Radiat. Res.* **10**, 449–461 (1959).
- ⁴ Manney, T. R., Brustad, T. & Tobias, C. A. *Radiat. Res.* **18**, 374–388 (1963).
- ⁵ Barendsen, G. W. *Int. J. radiat. Biol.* **8**, 453–466 (1964).
- ⁶ Deering, R. A. & Rice, R. Jr. *Radiat. Res.* **17**, 774–786 (1962).
- ⁷ Skarsgard, L. D., Kihlman, B. A., Parker, L., Pujara, C. M. & Richardson, S. *Radiat. Res. Suppl.* **7**, 208–221 (1967).
- ⁸ Todd, P. *Radiat. Res. Suppl.* **7**, 196–207 (1967).
- ⁹ Raju, M. R., Gnanapuri, M. & Richman, C. B. *J. Radiol.* **45**, 178–181 (1972).
- ¹⁰ Barendsen, G. W. in *The Initial Effects of Ionizing Radiations on Cells* (ed. Harris, R. J. C.) 183–194 (Academic, New York, 1961).
- ¹¹ Lawrence, J. & Tobias, C. in *Mod. Trends Radiother.* **1**, 260–276 (1967).
- ¹² Tobias, C. A. & Todd, P. in *National Cancer Institute Monograph 24, Conference on Radiobiology and Radiotherapy*, 1–15 (US Department of Health, Education and Welfare, 1967).
- ¹³ Sinclair, W. K. *Cancer Res.* **27**, 297–308 (1967).
- ¹⁴ Mortimer, R., Brustad, T. & Cormack, D. V. *Radiat. Res.* **26**, 465–482 (1965).
- ¹⁵ Puck, T. *Prog. Biophys. biophys. Chem.* **10**, 237–258 (1960).
- ¹⁶ Barendsen, G. W., Walter, H. M. D., Fowler, J. F. & Bewley, D. K. *Radiat. Res.* **18**, 106–119 (1963).
- ¹⁷ Chadwick, K. H. & Leenhouts, H. P. *Phys. med. Biol.* **18**, 78–87 (1973).
- ¹⁸ Todd, P. thesis, Univ. California, Berkeley (1964).
- ¹⁹ Todd, P. W. *Radiat. Res.* **61**, 288–297 (1975).
- ²⁰ Kapp, D. S. & Smith, K. C. *J. Bact.* **103**, 49–54 (1970).
- ²¹ Painter, R. B., Young, B. R. & Burki, H. J. *Proc. natn. Acad. Sci. USA* **71**, 4836–4838 (1974).
- ²² Cleaver, J. E., Thomas, G. H. & Burki, H. G. *Science* **177**, 996–998 (1972).
- ²³ Burki, H. J., Roots, R., Feinendegen, L. E. & Bond, V. P. *Int. J. radiat. Biol.* **24**, 363–375 (1973).
- ²⁴ Howard-Flanders, P. *Adv. Biol. med. Phys.* **6**, 553–603 (1958).
- ²⁵ Christensen, R. C., Tobias, C. A. & Taylor, W. D. *Int. J. radiat. Biol.* **22**, 457–477 (1972).
- ²⁶ Cole, A., Shonka, F., Corry, P. & Cooper, W. G. in *Molecular Mechanisms for Repair of DNA* (eds Hanawalt, P. C. & Setlow, R. B.) 665–676 (Plenum, New York, 1975).
- ²⁷ Ehmman, U. K. & Lett, J. T. *Radiat. Res.* **54**, 152–162 (1973).
- ²⁸ Cleaver, J. E. in *Meth. Cancer Res.* **11**, 123–165 (Academic, New York, 1975).
- ²⁹ Dugle, D. L., Gillespie, C. J. & Chapman, J. D. *Proc. natn. Acad. Sci. USA* **73**, 809–812 (1976).

Electrical demonstration of rapid light-induced conformational changes in bacteriorhodopsin

BACTERIORHODOPSIN (a purple pigment) is a light-driven proton pump^{1,2}, found in patches of the cell membrane of *Halo-bacterium halobium*^{3,4}, and is chemically similar to the visual pigment rhodopsin of animal eyes¹. In both molecules the chromophore retinal is linked as a protonated Schiff base to a lysine residue of the apoprotein opsin^{1,5,6}. Light induces bacteriorhodopsin to move protons from the inside to the outside of the cell. The resulting electrochemical proton gradient across the membrane is finally used to synthesise ATP. We report here the application of a new technique for the direct measurement of small capacitive charge displacements in oriented molecules. Our photoelectric measurements using asymmetrically aligned bacteriorhodopsin demonstrate

that this protein undergoes reversible conformational changes after absorption of light.

Flash spectroscopy shows that bacteriorhodopsin undergoes a cycle through at least five different spectroscopically defined intermediates⁷. About 8 ms after bleaching the pigment returns to its original form. This cycling time is in good agreement with the maximal 'proton pumping' rate at saturating light intensities. Measurements show that a single protein molecule can transport up to 100–200 protons per second⁸.

It is conceivable that the excited bacteriorhodopsin molecule, in addition, passes through a conformational transition sequence to accomplish the vectorial proton transport⁹. Such conformational changes are expected to generate charge displacements within the protein. To measure this effect electrically, two basic requirements have to be fulfilled. First, the molecules must be oriented in parallel but asymmetrically and second, they must be synchronisable, that is, they have to start with the change at a common zero time.

The most favourable system for fulfilling these conditions is found in photoreceptor cells of animals. Here, rhodopsin molecules are strongly oriented in lamellar cell structures, and synchronisation is achieved by means of short flashes. Fast capacitative photopotentials from a wide variety of photoreceptor cells have been measured, and have come to be known as 'early receptor potentials' (ERPs) (ref. 10). The signals are generally attributed to conformational changes within the rhodopsin molecule as it passes through its bleaching sequence¹¹.

We have succeeded in developing a model system in which rhodopsin from bovine retinal rod outer segments is reoriented on to one side of a thin Teflon film separating two aqueous phases¹². Flashes to the layer evoke fast photovoltages and photocurrents (depending on the measuring device). Since the layer, having virtually infinite resistance when arranged between two aqueous phases, can be considered as a capacitor, the observed photoelectric response is due to pure capacitative charge displacements. Such model layers offer, for instance, the advantage of allowing the measurement of true transition time courses unaffected by cell membrane time constants. We have now applied this approach to bacteriorhodopsin layers.

Surface-active material from the aqueous suspension of purple membrane fragments was obtained by agitation until foam formed. About 100 µl of the foam was transferred to one side of a two-compartment Teflon cell containing a 4 M NaCl subphase. Thereafter it was overlaid with hexane. The surface film in the water-hexane interface was apposed to a vertically mounted Teflon septum (6 µm thick) by slowly increasing the water level. The chamber on the other side of the septum contained only electrolyte. Thus, the model system consists of the spatial sequence: electrolyte–bacteriorhodopsin–Teflon–electrolyte. The Teflon septum between the two half-cells acts as an electrical insulator and couples the compartments only capacitatively. The two identical electrolyte solutions were connected by silver–silver chloride electrodes to a high resistance input electrometer ($10^{14}\Omega$; 20 pF) (ref. 12).

The voltage responses of this layer when illuminated with bright white flashes are illustrated in Fig. 1. The individual oscilloscope pictures originate from sequential flashes to the same layer. They differ in the time axis to delineate the full time course of the signal.

Analysing the photopotential time course for exponentials leads to, at least, three clearly distinguishable transitions: the signal increases with a fast time constant $\tau_1 \sim 1$ ms and a slower one, $\tau_2 \sim 15$ ms. The main decay back to the initial potential occurs with $\tau_3 \sim 1$ s. Correlating these time constants to those of the spectral intermediates is not feasible at the present time and with the present technique. In spite of this, the close agreement of τ_2 with the 412 nm→570 nm transition ($\tau \sim 12$ ms) is striking. Also, millisecond spectral transitions have been observed which could coincide with τ_1 (ref. 9).

Since bacteriorhodopsin undergoes a cyclic photoreaction, the photovoltage would be expected to be reversible; and indeed it is. The bleaching cycle in the purple membrane is

completed in approximately 10 ms, whereas in the model layer about a second is required. This discrepancy of two orders of magnitude is tentatively attributed to the different arrangement of the protein in the two systems: *in situ*, a lipid bilayer matrix, two sides of the protein contact aqueous media¹³; whereas *in vitro*, the protein is in contact with water only on one side—the more hydrophilic one. If a water-soluble reactant (presumably protons) is required for regeneration at the side where the bacteriorhodopsin molecule touches the Teflon surface, then the hindered accessibility would account for the inhibition of regeneration.

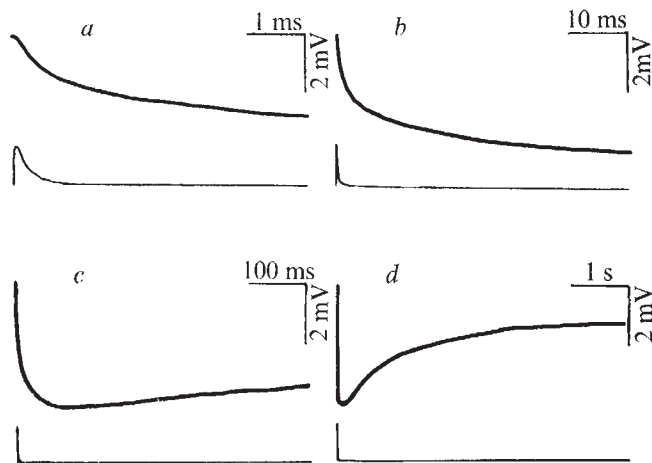


Fig. 1 Examples of the photovoltage of a bacteriorhodopsin layer, 6 mm in diameter, in response to white light flashes. Purple membrane fragments, prepared according to Oesterhelt and Stockenius¹⁴, were suspended in 3.0 ml basal salt sodium 4.35 M NaCl, 0.275 M KCl, 0.08 M MgSO₄, 9 mM Na citrate, pH 7.0) at a protein concentration of 0.5 mg ml⁻¹. The aqueous subphase contained 4 M NaCl and 10 mM imidazole adjusted to pH 7.1 with HCl. The experiments were carried out at room temperature (22 ± 1 °C). Each oscilloscope trace includes the time course of the flash at the bottom. Subsequent flashes were separated by more than 1 min. The following amplifier time constants were chosen: a, 1 Hz–10 kHz; b, 0.1 Hz–3 kHz; c, d.c.–1 kHz; d, d.c.–0.1 kHz.

The polarity of the voltage is negative on that side where the layer is formed. This is the expected polarity for protons moving from the aqueous phase to the bacteriorhodopsin layer. If this were so, then a strong increase in photovoltage amplitude should result from reducing the ionic strength. Furthermore, the time constants recorded above should depend on proton concentration. Changing the electrolyte (while the layer remains apposed to the Teflon), from 4 M NaCl to 4 mM NaCl and then back to 4 M NaCl, however, did not alter the photovoltage amplitude nor the time constants τ_1 , τ_2 , τ_3 , at any stage of the manipulations. Apart from stressing the stability of the bacteriorhodopsin layer this experiment rules out an origin of the photovoltage from a diffused ion double layer at the aqueous interface. More likely, the effect is based on charge or dipole reorientations in the bacteriorhodopsin molecule itself.

Changes of pH in the subphase at constant ionic strength did not significantly affect the time constants, τ_1 or τ_2 (range, pH 4–9). One effect of increased proton concentration is a faster decay of the photovoltage transient to zero. For instance, at pH 7, $\tau_3 = 2$ s and at pH 4, $\tau_3 = 0.5$ s. This gives further support to the above mentioned explanation for the slow regeneration rate: the increased proton concentration at lower pH compensates for the hydrophobic location of the hypothetical regeneration site.

At low pH the photovoltage amplitude increases slightly. A layer, which displays at pH 9 a maximal amplitude of 3 mV displays at pH 4 about 7 mV. At pH 3 the shape of the photovoltage response changes dramatically; the negative phase is

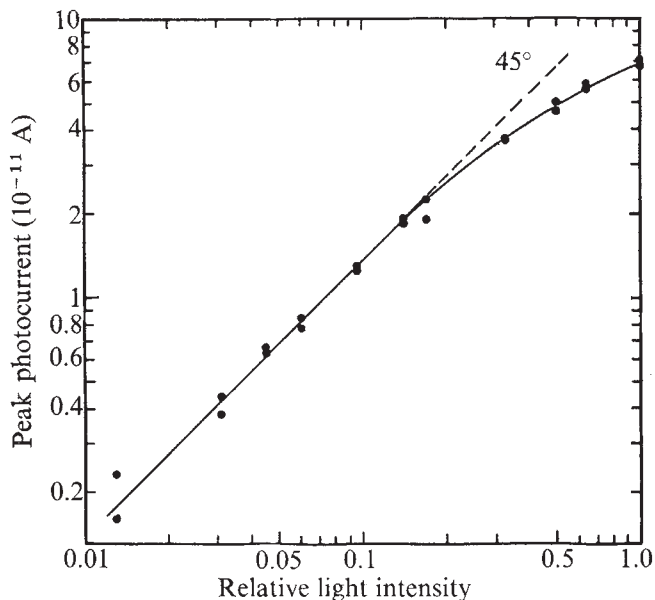


Fig. 2 Peak photocurrent as a function of the flash intensity. The increase in amplifier current time constant was limited to 1 ms. Neutral density filters were used to reduce the flash intensity. Data were obtained from one layer.

reduced in its amplitude and a very fast positive transient of 3 mV appears. The whole signal has a duration of 200 ms. These experiments indicate a proton contribution to the generating mechanism; since this is already localised in the protein (independent of ionic strength), the photovoltage or a part of it may directly reflect a proton movement in the protein molecule.

Since the bleaching of bacteriorhodopsin is reversible, it is possible to apply many flashes to the same layer, retrieving reproducible responses: one layer can be exposed a hundred times to a saturating flash without significant alterations of the photovoltage.

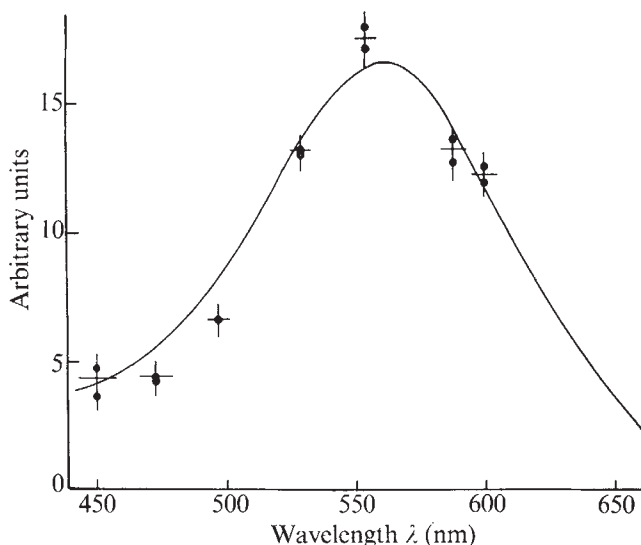


Fig. 3 Action spectrum of a bacteriorhodopsin layer. Solid line represents absorption spectrum of aqueous suspensions of bacteriorhodopsin. Each data point derives from the peak photocurrent at the given wavelength, normalised to the number of photons within the flash. Horizontal bars indicate the interference filter bandwidth at 50% transmission, and vertical bars represent the estimated experimental error.

The photoelectric effect can also be detected as a photocurrent if the electrodes are connected to an ammeter. In this case a photocurrent transient, which is exactly the first time derivative of the photovoltage, is measured. Therefore, the peak in the current transient reflects the steepest slope of the voltage. Since reducing the flash light intensity decreases the photovoltage amplitude, without affecting the shape, a dependence on light intensity could also be measured as the peak photocurrent amplitude. This method provides a better signal-to-noise ratio and is therefore used in the experiment illustrated in Fig. 2. For dim flashes the peak photocurrent is directly proportional to the light intensity. Brighter flashes lead to sublinear responses, indicating a saturation behaviour.

To further establish that the photoresponses result from bacteriorhodopsin bleaching, an action spectrum was obtained (Fig. 3), using the 'current' mode described above. The individual peak photocurrents lie without exception within the linear portion of the curve shown in Fig. 2. The absorption spectrum of the bacteriorhodopsin suspension used in the experiments (solid line) is fitted with its amplitude to the data points. The good agreement of both identifies bacteriorhodopsin as the molecular species responsible for the photoelectric effects.

We thank Dr N. Nelson (Department of Biology, Technion, Haifa, Israel) for bacteriorhodopsin. This work was supported by the Deutsche Forschungsgemeinschaft and CONACYT, Mexico.

H.-W. TRISSL*

M. MONTAL*†

Departamento de Bioquímica,
Centro de Investigación y de
Estudios Avanzados del
Instituto Politécnico Nacional,
México 14, DF, México

Received 20 December 1976; accepted 10 February 1977.

*Present address: Departments of Physics and Biology, University of California, San Diego, La Jolla, California 92093.

†Please address all correspondence to M.M.

- Oesterhelt, D., Stoekenius, W. *Proc. natn. Acad. Sci. U.S.A.* **70**, 2853–2857 (1973).
- Stoekenius, W. *Sci. Am.* **234**, 38–46 (1976).
- Oesterhelt, D. & Stoekenius, W. *Nature new Biol.* **233**, 149–152 (1971).
- Blaurock, A. E. & Stoekenius, W. *Nature new Biol.* **233**, 152–155 (1971).
- Lewis, A., Spoonhower, J., Bogomolni, R. A., Lozier, R. H. & Stoekenius, W. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4462–4466 (1974).
- Lewis, A. & Spoonhower, J. in *Neutron, X-Ray and Laser Spectroscopy in Biophysics and Chemistry* (eds Yips, S. & Chen, S.) (Academic, New York 1974).
- Lozier, R. H., Bogomolni, R. A. & Stoekenius, W. *Biophys. J.* **15**, 955–962 (1975).
- Oesterhelt, D. *Angewandte Chemie* **15**, 17–24 (1976).
- Slifkin, A. M. & Caplan, S. R. *Nature* **253**, 56–58 (1975).
- Cone, R. A. & Pack, W. L. in *Handbook of Sensory Physiology* 1 (ed. Loewenstein, W. R.) 345–365 (Springer, Heidelberg, 1971).
- Rüppel, H. & Hagins, W. A. in *Biochemistry and Physiology of Visual Pigments* (ed. Langer, H.) 257–261 (Springer, Heidelberg, 1973).
- Trissl, H.-W., Darszon, A. & Montal, M. *Proc. natn. Acad. Sci. U.S.A.* **74**, 207–210 (1977).
- Henderson, R., & Unwin, P. N. T. *Nature* **251**, 28–32 (1975).
- Oesterhelt, D. & Stoekenius, W. *Methods Enzym.* **31**, 667–678 (1974).

secondary amines⁴, but chlorogenic acid and 4-methylcatechol catalyse⁵ the nitrosation of piperidine at gastric pH. We report here that nitrosophenols can catalyse *N*-nitrosamine formation.

Nitrosophenols are formed by the nitrosation of phenols and their presence in cured smoked meats has been demonstrated⁶. The reaction we studied was that of 0.15 M sodium nitrite with 0.15 M pyrrolidine in citrate buffer, pH 5.0, at 37 °C. Perchloric acid was used to bring the pyrrolidine solution to pH 5.0 before mixing. The effect of *p*-nitroso-*o*-cresol on this reaction was studied at 0.25, 0.50, 0.75 and 1.0 mM concentration using the method of initial rates. Aliquots were taken from the reaction mixtures at 5-min intervals for 20 min. Each aliquot was quenched with a solution of ammonium sulphamate in 2 N H₂SO₄ to remove unreacted nitrite, and the nitrosopyrrolidine (NPY) was extracted into dichloromethane and determined by gas-liquid chromatography. Each reaction was repeated in triplicate. For each reaction a plot of NPY concentration against time gave a straight line, the slope of which gave the rate of formation of NPY. Figure 1 shows a plot of the rate of NPY formation against nitrosocresol concentration. Figure 2

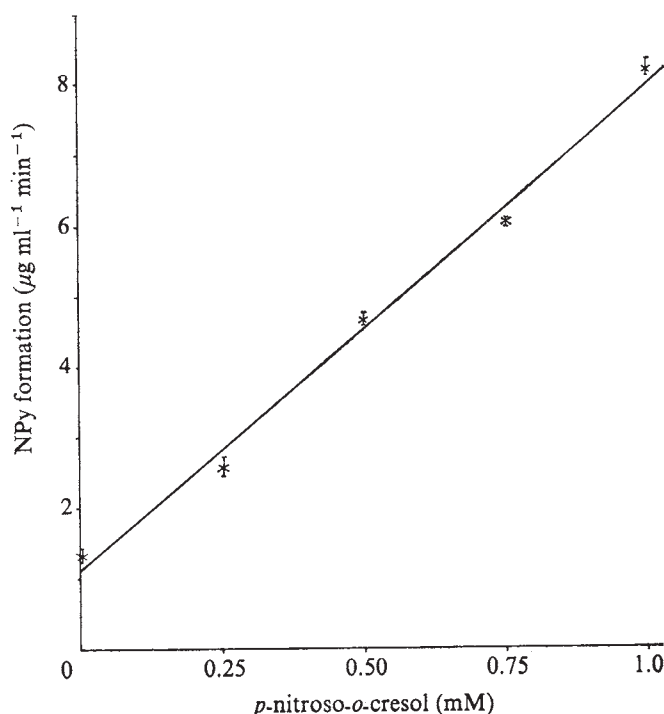


Fig. 1 Dependence of the rate of formation of *N*-nitrosopyrrolidine on the concentration of *p*-nitroso-*o*-cresol.

shows a plot of the logs of these two properties. That the points in Figs 1 and 2 do not fall exactly on straight lines may be due partially to the poor solubility of *p*-nitroso-*o*-cresol in the citrate buffer solution used. The slope of the line in Fig. 2 calculated by the method of least mean squares is 0.81, and this represents the order of the reaction with respect to *p*-nitroso-*o*-cresol. The two other nitrosophenols we have tested, *p*-nitrosothymol and *l*-nitroso-2-naphthol, also catalysed NPY formation at pH 5.0. No reaction takes place between *p*-nitroso-*o*-cresol and pyrrolidine at pH 5.0 in the absence of nitrite. The formation of NPY therefore does not occur simply by a trans-nitrosation process from nitrosocresol to pyrrolidine. We found that *N*-nitrosation does not occur in the organic phase during work-up by reduced nitrite species. Such nitrosamine formation requires the presence in the organic phase of nitrosating species and pyrrolidine. The acidic quenching procedure employed in these experiments, besides removing unreacted nitrite, will protonate unreacted amine. The protonated amine should remain in the aqueous layer during dichloromethane extraction. To check that this was so we prepared a 0.15 M solution

Catalytic effect of nitrosophenols on *N*-nitrosamine formation

THE effect of phenolic compounds on the rate of nitrosation of secondary amines is not a simple one. Tannins inhibit nitrosamine formation¹, but in suitable conditions gallic acid catalyses the nitrosation of diethylamine, the rate being dependent on pH and gallic acid concentration². Some phenolic constituents of smoked foods³ inhibit nitrosation of morpholine at pH 3.0. Phenolic compounds react with nitrite at a much faster rate than do

of pyrrolidine in pH 5.0 buffer, and put an aliquot through our quenching and extraction procedure. The organic extract was analysed for pyrrolidine in free and protonated forms. Free pyrrolidine was not detected, subject to a detection limit of $0.5 \mu\text{g ml}^{-1}$. The level of total pyrrolidine (free plus protonated) detected in the extract was $>1.0 \mu\text{g ml}^{-1}$. The amount of NPy that could be formed from this is insignificant compared to the levels being detected in the reactions. In a reaction mixture containing sodium nitrite (0.15 M), pyrrolidine (0.15 M) and *p*-cresol (0.075 M) in citrate buffer at pH 5.0 the concentration of NPy formed after 1 h was two and half times greater than that formed in a control reaction from which *p*-cresol was absent. It therefore seems that at suitable concentrations the presence of phenols can lead to an enhancement of the rate of nitrosamine formation.

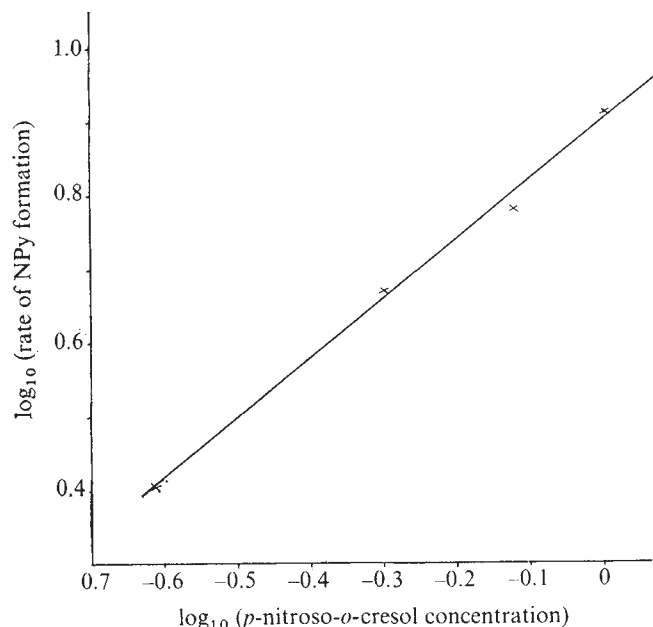


Fig. 2 Logarithms of the rates of formation of *N*-nitrosopyrrolidine plotted against the logarithms of the concentrations of *p*-nitroso-*o*-cresol.

Other kinetic data⁴ suggest that in a system containing nitrite, a phenol and a secondary amine, the phenol should be nitrosated at a faster rate than the secondary amine. The initial product of phenol nitrosation is the nitrosophenol, a relatively unstable species, which can be oxidised by atmospheric oxygen to the stable nitrophenol. In this system there are therefore two opposing effects on the rate of nitrosamine formation. The rate will be retarded by the phenol, but catalysed by the unstable nitrosophenol formed during the reaction by nitrosation of the phenol. The overall effect is likely to depend upon whether the steady-state concentration of nitrosophenol (as determined by the relative rates of its formation and its destruction by oxidation to nitrophenol) is sufficiently large to overcome the inhibitory effect of the phenol. In turn this will depend upon the relative degrees of retardation and catalysis of nitrosamine formation exerted by the phenol and the nitrosophenol respectively.

These observations elucidate the basis for some of the conflicting reports of the effects of phenolic compounds on the rate of formation of nitrosoamines, but the implications in terms of the formation of nitrosoamines in cured meats and *in vivo* are not clear. The effects of phenols at up to 300 mg kg^{-1} , which may be found in smoked meats (Knowles, M. E., unpublished) and of the 42–300 mg of phenolic material excreted daily into the stomach via gastric juice⁷ are likely to be much more complex. The complicating effects of other soluble and insoluble components and of adsorption effects in heterogeneous systems are not susceptible to quantitative prediction; our studies of the catalytic effect of

phenols which we have noted as being exerted through the formation of nitrosophenols are being extended to examine these additional factors.

We thank Mr Alan Young and Mr Colin Crews for technical assistance.

R. DAVIES
D. J. MCWEENY

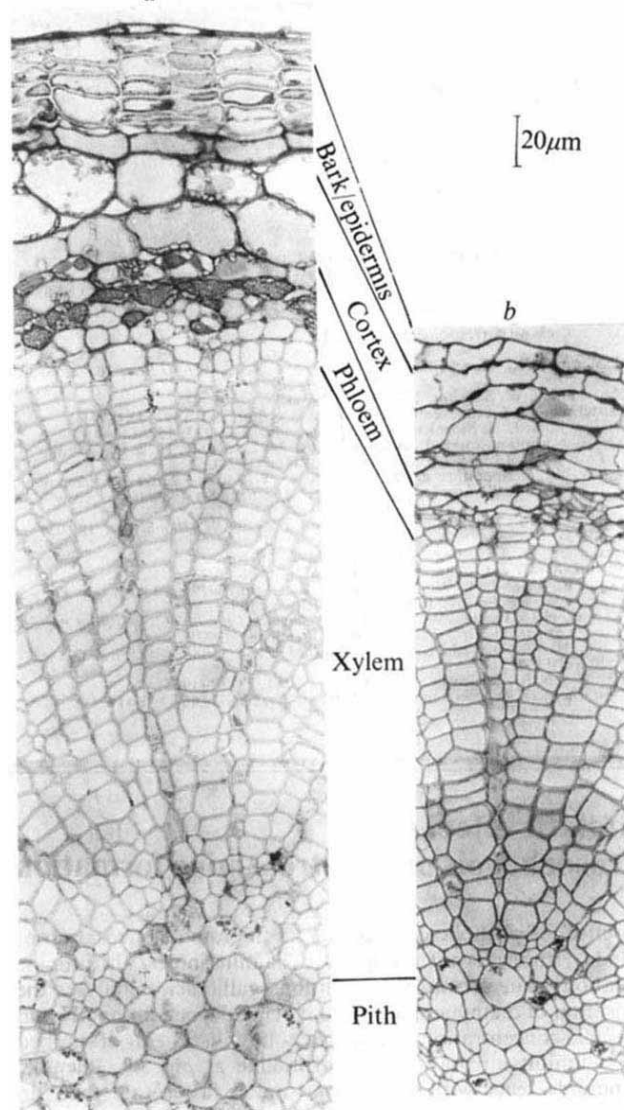
Food Science Division,
Ministry of Agriculture Fisheries and Food,
Food Laboratory (Colney),
Colney Lane,
Norwich, UK

Received 12 October 1976; accepted 25 February 1977.

- ¹ Bogovski, P., Castegnaro, M., Pignatelli, B. & Walker, E. A. *N-Nitroso Compounds Analysis and Formation*, No. 3, 127 (IARC Scientific Publications, Lyon, 1972).
- ² Walker, E. A., Pignatelli, B. & Castegnaro, M. *Nature* **258**, 176 (1975).
- ³ Issenberg, P. & Virk, M. *Abst. Pap. Am. Chem. Soc.* **168**, AGFD 53 (1974).
- ⁴ Challis, B. C. *Nature* **244**, 466 (1973).
- ⁵ Challis, B. C. & Bartlett, C. D. *Nature* **254**, 532–533 (1975).
- ⁶ Knowles, M. E., Gilbert, J. & McWeeny, D. J. *Nature* **249**, 672–673 (1974).
- ⁷ *Biochemist's Handbook* (ed. by Long, C.) 910 (Spon, London, 1961).

Erratum

In the article 'Stem anatomy and sheathing mycorrhizas in the *Betula verrucosa*–*Amanita muscaria* relationship' by P. A. Mason, J. Pelham and F. T. Last, *Nature* **265**, p. 334, Fig. 1b was inverted and is shown in correct relation to Fig. 1a below.



reviews

Comparative immunology

W. H. Hildemann

Comparative Immunology. By Edwin L. Cooper. Pp. 338. (Prentice-Hall: Englewood Cliffs, New Jersey, 1976.) \$19.95. £15.95.

THE evolutionary approach to cell-mediated and humoral immunity has gained increasing attention during the past decade. More biologists are eager to discover mechanisms and adaptive specialisations of immunocompetence because the survival of entire populations and species is involved. If mammals, including man, represent the quintessence of immunoresponsiveness with complex interdependent pathways, the less elaborate systems of immunity in lower phyla of animals offer insights of immediate biomedical interest. In a larger sense, wildlife conservation, management of food resources, and protection of ecosystems all hinge on understanding how diverse species cope with pathogenic or infectious agents. Seasoned biological scientists will find comprehensive summarisations of much work in this field in two recent volumes: *Immunologic Phylogeny* (ed. W. H. Hildemann and A. A. Benedict, Plenum: New York and London, 1975); *Phylogeny of Thymus and Bone Marrow-Bursa Cells* (ed. R. K. Wright and E. L. Cooper, North-Holland: Amsterdam, 1976).

In essence, different levels of immunorecognition and reaction to foreign agents are now discernible in phylogenetic progression all the way from coelenterates to mammals. The immune systems of higher vertebrates reveal specialisations of more general systems of receptors and mediators. Moreover, both immunological specificity and memory associated with leukocytic cells have been demonstrated in various invertebrate phyla, even though production of immunoglobulin antibodies first appears in vertebrates (that is, primitive fishes). Highly polymorphic systems of histocompatibility markers demonstrable as cell-surface alloantigens seem to be universal in Metazoa at all levels of phylogeny. These specificities evidently constitute immunorecognition molecules of exquisite discrimination in lower invertebrates sufficient to preclude the need for an additional system of immunoglobulin receptors. These two systems apparently function cooperatively in the separate T-lymphocyte and B-lymphocyte pathways of higher vertebrates.

Despite much progress, many in-

formation gaps and uncertainties remain at all levels of phylogeny. Until now, potential newcomers to comparative immunology have had no elementary reference to study, because the many immunology books available all deal almost entirely with placental mammals and man in a medical context. Ed Cooper has successfully pioneered in writing, according to his preface, "a unique beginning text for advanced undergraduate students of biology, zoology, and immunology . . ." The first four chapters provide a far-ranging introduction to the immune system and its phylogeny at a moderately advanced level. The subject matter anticipates later chapters and presupposes substantial understanding by the student of zoology, genetics and biochemistry.

Chapters 5, 6 and 11 focus in admirable breadth on invertebrate immune responses, a frontier subject the author handles with incisive enthusiasm. Only the recent discoveries of immunocompetence in corals and intraspecific incompatibilities in sponges are notably missing. Many immunologists would contest Cooper's assertion that even unicellular protozoa show quasi-immunorecognition. Indeed, the existence of specific immunocompetence in higher invertebrates has only recently gained reluctant acceptance. Although some may fault Cooper's protagonist stance in this connection, readers should enjoy the privilege of judging the evidence presented for themselves.

Chapters 8, 9 and 10 together cover transplantation immunology from both phylogenetic and ontogenetic viewpoints, with appropriate emphasis on the classes of non-mammalian vertebrates. More

attention could well have been given to the major histocompatibility complexes of mice (*H-2*) and men (*HLA*), although other excellent books emphasising these Immunogenetic systems (for example, J. Klein, *Biology of the Mouse Histocompatibility-2 Complex*, Springer: New York, 1975, and G. Snell, J. Dausset and S. Nathenson, *Histocompatibility*, Academic: New York and London, 1976) have recently appeared.

Chapters 12 and 13 deal with antibody synthesis and the immunoglobulins, respectively. These inseparable topics might better be considered earlier by many students, either along with chapter 7 on the "Machinery of the Immune System" or after the four introductory chapters. In the final chapter, Cooper presents a thoughtful epilogue focusing on unresolved questions concerning adaptive immunity. Beyond pedagogic questions of chapter sequence, the book suffers slightly from a number of poorly reproduced illustrations and an index with entries too abbreviated to identify topics with desirable precision.

All things considered, I wholeheartedly recommend *Comparative Immunology* as a widely useful reference for immunologists and zoologists alike. It can serve quite well as an immunobiology textbook for advanced undergraduates, or even graduate students other than dental, medical or public health students. Professor Cooper has made a specially valuable contribution to the literature of immunology. □

W. H. Hildemann is Professor of Immunology and Immunogenetics at the University of California, Los Angeles, California.

Organic photochemistry

Aspects of Organic Photochemistry. By W. M. Horspool. Pp. viii+290. (Academic: London and New York, 1976.) £9.50; \$20.75.

THIS book sets out to bridge the gap between the many recent introductory texts on organic photochemistry and the more comprehensive books and reviews. It succeeds to a certain extent, providing three chapters of introductory material (Introduction to physical aspects of photochemistry, Experimental methods, Orbital symmetry correlations) and six chapters

of more detailed organic photochemistry. The later chapter headings (Photochemistry of unsaturated systems, Photochemistry of aromatic compounds, Reactions of ketones, Photochemistry of enones, Oxidation and reduction reactions, Miscellaneous reactions) resemble the divisions used in the Chemical Society's Specialist Periodical Reports, *Photochemistry*, but whereas this presentation is appropriate for a specialised annual survey it is less so for a textbook intended in the first place for final-year undergraduate and graduate students. A

complete commitment to a description based on functional groups would have avoided the separation of topics which can best be appreciated or taught together (for example, Norrish type II reactions and photoreduction of ketones).

The chapter on experimental methods is particularly welcome, highlighting the ways in which the practice of photochemistry differs from that of 'ordinary' chemistry. The other introductory chapters are weaker, which is a pity when they are meant to give a helpful background for the later chapters. Throughout the book there is an accumulation of loose phrasing and of errors (of the type: (pp5, 6 and 9) the impression is given that the oxygen atom of formaldehyde has only one lone pair; (p155) it is incorrectly stated that there are eight

π orbitals in a benzyl-type anion; (p193) the reaction (21)→(22) is not, as stated, a vinylogous Norrish type I reaction) which could confuse the reader. Another distracting feature is the use of four different ways of indicating chemical reactions—by structural formula number, by figure number, by equation number, and by no reference at all (on occasion where the equation and the relevant text are a page apart).

For the organic photochemist the book could be a useful complement to others on his shelf (though it lacks references to primary literature), but I would hesitate to recommend it for teaching purposes.

J. D. Coyle

J. D. Coyle is Senior Lecturer in Chemistry at The Polytechnic, Wolverhampton, UK.

Biological rhythms

An Introduction to Biological Rhythms. (Tertiary Level Biology series.) By D. S. Saunders. Pp. viii+170. (Blackie: Glasgow and London, 1977.) £5.50.

THIS book introduces the reader to a wide variety of aspects of circadian rhythms: to their endogeneity, their entrainment by a variety of complex patterns of light or temperature, their implication in the control of reproductive and other rhythms by day-length—photoperiodism—and their function in navigation. It also considers rhythms with other periods—tidal, lunar or annual. All these are well described.

The author's particular interest in insects is rather too prominent: those aspects of rhythms manifested by insects are very fully discussed, with at times considerably more detail than one might expect of the advanced undergraduate student; data from other animals and from plants are included when they manifest essentially the same phenomena as can be seen in insects, but not often otherwise. Thus the fascinating problem of the hierarchical or other organisation between the presumed multiple oscillators in vertebrates receives the briefest mention, although the organisation in insects, with an apparent transmission from a clock in the central nervous system by way of either action potentials or neuro-humours, receives considerable attention. Population rhythms, rhythms in events which each individual encounters only once, are discussed in great detail with regard to the eclosion of insects; the similar mammalian examples, such as circadian rhythm in the time of birth or death, escape mention, although admittedly they are less amenable to study.

The choice and production of the figures is not always happy. A single figure frequently extends over two or more pages, and by omitting essential data they often confuse rather than enlighten. The section on further reading would be improved by more reference to symposia and reviews; it is unfortunate that the very valuable symposium in the October 1976 issue of *Fedn Proc.* appeared too late for mention. Perhaps the worst feature of the book, especially for a student text, consists of two frankly erroneous and hardly credible statements about the length of the solar day which appear on the first page; these first 9 pages are in any case largely superfluous.

J. N. Mills

J. N. Mills is Brackenbury Professor of Physiology at the University of Manchester, UK.

Research on chloroplast metabolism

The Intact Chloroplast. Edited by J. Barber. Pp. xi+476. (Elsevier: Amsterdam, New York and Oxford, 1976.) Dfl.140.

THIS book is intended to be the first of a series presenting reviews by specialists in different areas of photosynthesis research. The reviews are designed to provide an up-to-date account of research on their subject, while at the same time providing sufficient background material, and information about the methods used, to allow readers working on other aspects of photosynthesis, plant physiology, or bioenergetics to understand the results and apply them to their own research or teaching.

This volume deals, in most of the chapters, with the interactions between the different chloroplast compartments, or between the chloroplast and the remainder of the cell. This work has only become possible since the development of techniques for the isolation of intact chloroplasts, and represents a reversal of the classical biochemical trend towards the use of simpler systems. The effects which the use of this organelle-based approach is having on many aspects of photosynthesis research are well documented.

An introductory chapter on chloroplast structure by Coombs and Greenwood is followed by chapters on ion transport and energy conservation, (Vredenberg, Barber), ATP synthesis (Hall), the energetics of carbon metabolism and its relationship to transport of materials

between chloroplast and cytoplasm (Krause and Heber, Walker, Heldt, Coombs), sulphate metabolism (Schwenn and Trebst) and protein and amino acid biosynthesis (Ellis, Leech and Murphy). In the final chapter Raven attempts an overall view of the energetics of photosynthetic growth.

On the whole the authors have succeeded in presenting the material clearly and intelligibly, although one or two present an excess of undigested data. There is also some overlapping of subject matter between a number of the chapters on metabolite transport systems, both within the book, and with recent reviews published elsewhere. The book succeeds, however, in demonstrating the rapid changes taking place in our understanding and attitude to the relationship between chloroplasts and the remainder of the organism. It also illustrates one of the failures of photosynthesis research, the relationship between ATP synthesis and electron transport. Twenty-five years after the discovery of photophosphorylation it is not known how much ATP is synthesised per electron transported, or how it is made. It is also unclear whether "cyclic photophosphorylation" exists; from this book it seems that physiologists think it does occur, and biochemists think it does not.

The book can be recommended for any library dealing with photosynthesis research or advanced teaching as an authoritative source of information on the present state of research on chloroplast metabolism.

M. C. W. Evans

M. C. W. Evans is Reader in Plant Chemistry in the Department of Botany and Microbiology at University College, London, UK.

Blood-brain barrier

Blood-Brain Barrier in Physiology and Medicine. By Stanley I. Rapoport. Pp. xii+316. (Raven: New York, 1976.) \$28.

THE concept of the blood-brain barrier derives from the classical studies on intravital staining carried out by Ehrlich and Goldmann early in this century; they observed that parenteral injections of acidic dyes, such as Trypan blue, resulted in staining of connective tissues but not of central nervous tissue. Until the mid-1950s the concept remained rather vague and its reality was often called into question, in spite of several quantitative studies, mainly from Brodie's laboratory.

In 1956 the reviewer published his *Physiology of the Ocular and Cerebrospinal Fluids*, a book in which the dynamic aspects of exchanges within the central nervous system were related to current concepts of transport in biological systems; ten years later his *Physiology of the Cerebrospinal Fluid* integrated the considerable accession of knowledge that probably resulted from the stimulus to research in the field of brain transport provided by his first monograph. More than ten years have elapsed since the publication of this latter book, and during this period not only has research been proceeding rapidly in the field of brain transport but also several monographs and symposium volumes have been published that have amplified and brought up to date many of the aspects covered by the *Physiology of the Cerebrospinal Fluid*.

Rapoport's *Blood-Brain Barrier* may be looked at in this context; the basic ideology and many of the experimental facts were already familiar before he began to write the book, and it is interesting to consider how he has approached his task and with what success. After a brief introduction to cell structure, the second chapter presents, in lucid fashion, the basic elements of transport across cell membranes, proceeding from the sample case of a single cell to the more complex one of transport across epithelial layers. In the next chapter quantitative aspects of the barrier between blood and extracellular space of the brain are dealt with, and the site of the barrier is, in accordance with recent studies of Reese and Karnovsky, definitely attributed to the capillary endothelium, which differs from that of connective tissue in having its intercellular clefts sealed by tight junctions.

In the next chapter the quantitative aspects of brain capillary permeability are analysed in the light of existing information; this is an especially valuable chapter, in which the author describes his own recent studies on the 'opening of the barrier' by preliminary treatment of brain tissue with hypertonic solutions, a

treatment that reversibly seems to open up intercellular junctions that had been tight. The chapter also includes a discussion on brain oedema, a discussion that, in the reviewer's opinion, betrays a defective understanding of the importance of solute flow across the barrier. In connective tissue, oedema follows from a reduction of the colloid osmotic pressure of the plasma or from an increase in that in the extracellular spaces, in accordance with the Starling principle. The flow of fluid in response to altered colloid osmotic relations is feasible because of the absence of significant restraints on the passage of the non-colloidal dissolved constituents of plasma. In the brain, however, this is not true, so that a mere alteration in colloid distribution can have little effect on the water distribution between blood and tissue.

Two chapters deal with pathology of the barrier and the regulation of drug entry; this latter seems unnecessary as a

separate chapter since the facts belong to the general subject of barrier permeability. The same may be said of the following chapter, which deals with transport of sugars, amino acids and 'other substances'. The chapter is valuable in summarising our knowledge but should have followed immediately on the earlier description of kinetics.

In the final chapter the author follows the reviewer's precedent and brings the barriers in the eye in relation to those in the brain; especially interesting are the phenomena of 'osmotic opening' of the barriers in the eye.

Rapoport has provided a very useful book that gives a clear view of the basic phenomena and their interpretation to the uninitiated, and provides a summary of recent work that is valuable to the established worker in the field.

Hugh Davson

Hugh Davson is Honorary Research Fellow at King's College, University of London, UK.

Colourful chemistry

Colour and Constitution of Organic Molecules. By J. Griffiths. Pp. ix+281. (Academic: London and New York, 1976.) £9.50; \$20.75.

To a colour chemist the absorption spectra of organic molecules between wavelengths of 400 and 700 nm is a matter of overriding importance. To an organic chemist it is a relatively narrow part of the whole spectral range in which electronic transitions can be detected. Technologically, dyestuffs have important properties that distinguish them as a class amongst organic molecules, but the principles underlying the interpretation of their spectra are the same as for any colourless organic molecule that absorbs in the ultraviolet region of the spectrum.

In this book, John Griffiths provides a brief but useful review of the molecular orbital method at both the Huckel and semi-empirical SCF level. These theories, in so far as they are used to interpret organic electronic spectra, are a product of the 1950s. The all-electron methods developed later have had little impact on the subject, perhaps because of the success of these earlier theories. The book also contains a brief account of the theory of band intensities, lifetimes and the Franck-Condon envelope.

The crux of the book, its *raison d'être*, is a suggested classification of coloured organic compounds into "four broad classes". These are $n \rightarrow \pi^*$ chromogens, donor-acceptor chromo-

gens, cyanine-type chromogens, and acyclic and cyclopolyene chromogens. The last class is rather broad and is discussed under four sub-classes, acyclic polyenes, polycyclic benzenoid compounds, non-benzenoid alternant cyclic systems and non-alternant systems. This type of classification may be new to colour chemists but it closely follows the pattern of chapters in several books of organic spectroscopy.

The part of the book which comes closest to providing some analysis of organic electronic spectra which is not found in other, more comprehensive, texts is that which deals with donor-acceptor chromogens. The importance of this part of the book can be seen by the statement: "with the exception of the polycyclic quinones and the phthalocyanines, all the commercially important synthetic dyes are of this type". This is a good account with reference to several important papers. I was, however, surprised to see no acknowledgment to the work on inter- and intramolecular charge-transfer spectra of Mulliken, Nagakura and others. The absence of an author index prevented me from checking that this acknowledgment was not hidden in some other part of the book.

John Griffiths has written a useful book. I do not believe that it contains much that will be new to those whose interest has been the wider subject of the electronic spectra of organic compounds but it may encourage more colour chemists to discover the theoretical advances that have been made in this field.

J. N. Murrell

J. N. Murrell is a Professor of Chemistry at the University of Sussex, UK.

Frontier molecular orbital theory and organic reactions

Frontier Orbitals and Organic Chemical Reactions. By Ian Fleming. Pp. vii+249. (Wiley-Interscience: London and New York, 1976.) £3.95; \$8.50.

ORGANIC CHEMISTS have traditionally used resonance theory and handy 'arrow-pushing' methods of electron book-keeping to rationalise their reactions, or to predict new results. Although organic chemists used Hückel's model to predict stable and unstable cyclic conjugated systems, molecular orbital theory was not generally used as a qualitative predictive tool until Woodward and Hoffmann devised the classic orbital symmetry selection rules for concerted reactions in 1965. Useful in themselves, these rules also profoundly influenced the way organic chemists think about reactions. The work of Evans in the 1930s, of Dewar in the 1950s, and of Fukui in the 1950s and 1960s, was dusted off and assimilated by organic chemists. Fukui proposed in 1952 that the shapes and energies of the frontier molecular orbitals (FMOs)—the highest occupied MO (HOMO) and lowest unoccupied MO (LUMO)—of a molecule were crucial in determining the reactivity of a molecule. Successful applications of this idea to most areas of organic reactivity have led to the reformulation of theories of organic reactivity in terms of approximate quantum mechanical principles.

Fleming's book is the first general introduction to, and review of, the application of FMO theory to organic reactions. The book is unadorned by mathematics, and will be enormously successful in its goal of educating chemists at all levels of preparation in the use of this powerful qualitative theory. Fleming's approach is to show how FMO theory can deal with all of the subjects that fascinate organic chemists—rates, products, and stereoselectivities of reactions. He begins by developing the general idea of MOs and bonding, building up quickly to the valence MOs of ethane. Realistically, one would expect that some more facility than the expected 'familiarity with the concept of a molecular orbital' would be needed by the reader to fully understand this section, but other books are available to fill this need. After this introduction, Salem's and Klopman's treatments of intermolecular interactions, which include interactions other than FMO interactions, are briefly discussed, and the limitations of FMO theory are pointed out. FMO theory is more than an approximation to a full treatment of intermolecular reactions, because it is becoming increasingly apparent that FMO interactions are even more important in transition states that they seem to

be on inspection of isolated reactant orbitals.

Fleming continues with substantial chapters on ionic, pericyclic, radical, and photochemical reactions. Included in these chapters are generalisations about the FMOs of donor, acceptor, and conjugated alkenes, and excellent discussions of aromatic substitutions, ambident reactivity, the HSAB principle, and Woodward-Hoffmann rules. In the desire to make the theory explain everything, Fleming occasionally does a bit of hand-waving. For example, the ambident reactivity of delocalised radicals and anions cannot be fully explained by FMO theory alone, let alone be accurately predicted. I share Fleming's confidence in the theory, but others might be surprised to find him deducing the shapes of FMOs of molecules such as methylmaleic anhydride from products of reactions. The attitude of this book is that FMO theory is incredibly powerful, even if it must occa-

sionally be bent somewhat to fit with experience. Indeed, FMO theory is a MO-based conceptual framework on which the vast experience of organic chemistry can be organised.

I counted six insignificant typographical errors, and only one of any consequence. The calculation of periselectivity of hexatriene dimerisation on p166 is incorrect due to the incorrect placement of summation signs, so that the correct prediction is accidentally made. FMO theory seems to be charmed, indeed!

The book is organised so well, and contains so much factual information, as well as good insight and interesting speculation, that it creates many ideas for research and experimental tests. I heartily recommend the book to chemists interested in understanding organic reactions.

K. N. Houk

K. N. Houk is Professor of Chemistry at Louisiana State University, Baton Rouge, Louisiana.

Spin labels

Spin Labeling Methods in Molecular Biology. By G. I. Likhtenshtein. Translated by Philip S. Shelnitz. Pp. xiii+258. (Wiley-Interscience: New York and London, 1976.) £26; \$39.

ONE can read a translation like this with two purposes in mind. First, one might be hoping to learn about electron spin resonance (ESR) and the way that it can be applied to the problems of molecular biology. Second, one may be reading with the particular purpose of finding out more about the studies by Russian workers, published in less familiar or untranslated journals. This book is generally interesting on both counts.

The approach adopted is very much an empirical one. Theoretical discussion is limited to that absolutely necessary for the interpretation of the experimentally obtained spectra. The first chapter is largely concerned with presenting a series of empirical relationships between spectral shape and the motional parameters for spin labels. This in fact makes a very good introduction to spin labelling for the molecular biologist, since biological studies are very largely empirical, with relatively little necessity (at least at present), for detailed spectral stimulation.

The second chapter is concerned with the double label technique, in which two spin labels are introduced into an enzyme to determine the distance between the two functionally labelled groups. The technique can also be used to determine the distance between a spin label on a protein and a paramag-

netic ion in solution or bound to the protein. The result is, of course, a distance from a spin label group rather than from a group of the native protein, but problems of this kind dog any attempt to use probe molecules to obtain detailed structural information about proteins. All that can be said is that the distances obtained using such techniques agree well with those obtained with native proteins using X-ray diffraction.

The effects of conformational changes on spectra are then described, and a discussion is presented of the importance of the local mobilities detected by ESR for general theories of enzyme activity. Chapters are also included on applications of spin labels to problems of membrane and nucleic acid structure and on the effects of spin labels on nuclear magnetic resonance spectra. Finally, the spin label technique is compared with the possibilities of other probe methods.

All in all, the book provides a very useful guide to the style of spectral interpretation actually used by most molecular biologists. As a translation, it is also fairly successful, although it does contain a few gems. So we read of spin labels being "reduced with great intensity", of spin labels being "implanted" on to proteins and of EPR which "show a fine reaction to the approach of other paramagnetic groups". And how about this for sheer impenetrability: "Nitroxide radicals are reduced to hard to accommodate N-hydroxylamines" (p28).

A. G. Lee

A. G. Lee is Lecturer in the Department of Physiology and Biochemistry, University of Southampton, UK.

announcements

Awards

Glaxo is to offer annual awards totalling £16,000 to science writers throughout the EEC. Two major awards will be offered in each country—one Fellowship worth £1,000 for the best article or series of articles on a science subject and one Fellowship worth £1,000 for the best script and/or radio or television programme on a science subject. The Fellowships will be awarded to science writers who, in the opinion of the judges, have consistently done most to enhance the quality of science journalism. UK Fellowships will be administered by the Association of British Science Writers and the two £1,000 UK Awards for 1977 will be based on work published or broadcast during last year.

Details of the 1977 Fellowships to be offered in the UK from: Association of British Science Writers, c/o Glaxo Holdings Ltd, Clarges House, 6-12 Clarges Street, London, W1.

Meetings

25–29 April, **11th International Symposium on Remote Sensing of Environment**, Ann Arbor (Conferences and Institutes, 412 Maynard Street, Ann Arbor, Michigan 48109).

25 April, **Noise in our Society**, Edinburgh (The Royal Society of Edinburgh, 22/24 George Street, Edinburgh).

27 April, **Annual General Meeting of The Royal Meteorological Society**, London (T. M. R. Whitwell, James Glaisher House, Grenville Place, Bracknell).

5 May, **Carcinogenesis in Man**, Edinburgh (The Royal Society of Edinburgh, 22/24 George Street, Edinburgh).

13 May, **Agriculture and Forestry in the Uplands of Scotland**, Edinburgh (The Royal Society of Edinburgh, 22/24 George Street, Edinburgh).

3 June, **Clinical and Experimental Applications of Krypton 81 m**, London (The British Institute of Radiology, 32 Welbeck Street, London W1).

25–28 July, **Synthesis in Organic Chemistry**, Oxford (The Chemical Society, Burlington House, London SW1).

13–14 July, **Rapid Analysis in the Metallurgical Industry**, Sheffield (Institute of Metallurgical Technicians, Northway House, Whetstone, London N20).

25–29 July, **Solar Building Technology**, London (J. Chapman, North East London Polytechnic/UNESCO, Forest Road, London E17).

11–13 August, **Annual Meeting of the American Society of Pharmacology**, Washington, (L. R. Brady, Pharmacy, University of Washington, Seattle, Washington 98195).

15–20 August, **6th European Malacological Congress**, Amsterdam (Congresbureau van de Vrije Universiteit, De Boelelaan, 1105, Amsterdam).

8–9 September, **Annual Meeting of the British Society for Cell Biology**, Cardiff (J. F. Watkins, Medical Microbiology, Welsh National School of Medicine, Heath Park, Cardiff).

Person to Person

Exchange of furnished four bedroomed house in Guelph, Ontario for similar in Aberdeen area from August 1977 to July 1978. Contact David Piggins, Department of Psychology, University of Guelph, Ontario.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

12–15 September, **7th European Solid State Device Research Conference**, Brighton (The Institute of Physics, 47 Belgrave Square, London SW1).

12–14 September, **EMAG 77 (Developments in Electron Microscopy)**, Glasgow (The Institute of Physics, 47 Belgrave Square, London SW1).

26–29 September, **1st BOC Prestitley Conference—Heterogeneous Oxidation**, Leeds (J. T. Gleave, University of Leeds).

17–19 October, **17th Hanford Biology Symposium**, Washington (J. A. Rising, Biology 331 Bldg, Battelle—Northwest, Richland WA 99352).

23–26 October, **27th Canadian Chemical Engineering Conference**, Calgary (J. Higgins, 304 Examiner Bldg, 805 5th Street SW, Calgary, Alberta, Canada).

31 October–4 November, **Water Chlorination: Environmental Impact and Health Effects**, Gatlinburg, Tennessee (R. L. Jolley, Union Carbide Nuclear Division, PO Box Y Oak Ridge, Tennessee 37830).

31 October–2 November, **International Symposium on Sexually Transmitted Diseases**, Montreal (R. Morisset, 1650 Lincoln, Suite 1806, Montreal, Quebec).

4–6 January 1978, **15th Annual Solid State Physics Conference**, Coventry (The Institute of Physics, 47 Belgrave Square, London SW1).

4–7 January 1978, **International Symposium on Biomolecular Structure, Conformation, Function, and Evolution**, Madras (R. Srinivasan, Physics, University of Madras, Madras-600025, India).

Reports and Publications UK & Ireland—February

Department of Industry: Committee on Corrosion. Controlling Corrosion. 1: Methods. Pp. 16. 2: Advisory Services. Pp. 16. (London: Secretary, Committee on Corrosion, Department of Industry, Abell House, John Islip Street, SW1, 1976) *gratis*. [12]

Department of the Environment: Central Unit on Environmental Pollution. Environmental Mercury and Man. (A Report of an Inter-Departmental Working Group on Heavy Metals.) (Pollution Paper No. 10.) Pp. viii+92. (London: HMSO, 1976.) £1.40 net. [32]

Universities Research Reactor. Annual Report, 1975/1976. Pp. iii+42. (Risley, Warrington: Universities Research Reactor, 1977.) [32]

Proceedings of the Royal Irish Academy. Vol. 76, Section A. Nos. 14, 15 and 16: Torsion Theoretic Results in Procategories, I, II, III. By T. Porter. Pp. 145–172. £1.20. No. 17: C-Sets in Finite Groups. By D. MacHale. Pp. 173–180. 45p. No. 18: Some Characterisations of Shrinking Decompositions in Locally Convex Spaces. By P. K. Kamthan and S. K. Ray. Pp. 181–190. 48p (Dublin: Royal Irish Academy, 1976.) [42]

Scottish Development Department: Engineering Division. Applied Research and Development Report No. ARD 3: Development of a Water Quality Index. Pp. vii+62 (Edinburgh: Engineering Division, Scottish Development Department, 47 Robb's Loan, 1976.) *gratis*. [42]

Department of Energy. Energy Saving in the Home. Pp. 5. Compare Your Home Heating Costs: An Independent Guide to the Costs of Different Fuels and Heating Methods and How to Reduce Them. Pp. 22. (London: Department of Energy, Thames House South, Millbank, SW1, 1977.) *gratis*. [72]

Ministry of Agriculture, Fisheries and Food. Fishery Investigations, Series II, Volume 28, No. 1: Settlement, Growth, and Production of the Mussel, *Mytilus edulis* L., in Morecambe Bay, England. By P. J. Dare. Pp. iv+25. (London: HMSO, 1976.) £1.70 net. [72]

University of Newcastle Upon Tyne. Careers Advisory Service—Annual Report of the Chief Careers Adviser, 1975/76. Pp. 10. (Newcastle Upon Tyne: The University, 1977.) [92]

Institute of Cancer Research, London. Serial Mortality Tables. Neoplastic Diseases Vol. 1: England and Wales, 1911–70. Pp. 141. Neoplastic Diseases Vol. 2: Ireland (Republic), 1922–70. Pp. iii+142. Neoplastic Diseases Vol. 3: Northern Ireland, 1922–70. Pp. iii+142. Neoplastic Diseases Vol. 4: Scotland, 1911–70. Pp. iv+140. Deaths and Death Rates by Sex, Age, Site and Calendar Period. (London: Division of Epidemiology, Institute of Cancer Research, 1976.) [92]

Britain in Space. (Capabilities and Products of Member Companies.) Pp. 31. (London: The United Kingdom Industrial Space Committee, 29, King Street, St. James's, SW1, 1977.) [112]

The Year Book of the Royal Society of London, 1977. (No. 81). Pp. 421. (London: The Royal Society, 1977.) UK £3.50; Overseas £3.60. [142]

The Collection and Reduction of Gravity and Magnetic Survey Data. By C. McCann. (Computer-Based Packages for Teaching Earth Sciences—Report No. 2. Reading University Geological Reports No. 8.) Pp. 49. (Reading: Geology Department, The University, 1976.) [142]

Environmental Impact Analysis. A study prepared for the Secretaries of State for the Environment, Scotland and Wales by J. Catlow and C. G. Thirlwall. Pp. 116. (Research Report 11.) (London: Department of the Environment, 2 Marsham Street, SW1, 1976.) [142]

The Elgin Reptiles. By Michael Benton. Pp. 20. (Elgin, Moray: Moray Society, 1 High Street. Available from Elgin Museum, 1 High Street, Elgin.) 20p. [152]

National Radiological Protection Board. NRPB-R44: Concentration of Actinides in the Food Chain. By R. A. Bulman. Pp. 48. (Harwell, Didcot, Oxon.: National Radiological Protection Board, 1976.) [152]

- Otter Sprint Analysis. By Jean B. Webb. Pp. 12. (An Occasional Publication of the Mammal Society. (Reading, Berks: Mammal Society, 62 London Road, 1977. Available from the Society's Publications Sales Agent, "Larkmead", Barton Mills, Bury St. Edmunds Suffolk, 60p plus 10p p&p. [162]
- The British Hydromechanics Research Association. Twenty-ninth Annual Report. Pp. 42. (Cranfield, Bedford: The British Hydromechanics Research Association, 1977). [162]
- Department of Energy. Fuel Efficiency Booklet No. 8: The Economic Thickness of Insulation for Hot Pipes. Pp. 40. (London: Department of Energy, 1977). [172]
- International Planned Parenthood Federation. Programme Review and Financial Statements 1975 and 1976. Pp. 15. Occasional Essay No. 1: Some Ethical Issues in Family Planning. By Fred T. Sai. Pp. 36. (London: IPPF, 18-20 Lower Regent Street, SW1, 1976 and 1977). [172]
- Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 276. No. 950: Form and Evolution in the Anomacea (Mollusca: Bivalvia)—*Pododermis*, *Anomia*, *Patro*, *Enigmionia* (Anomia): *Placunanomia*, *Placuna* (Placunidae: Fam. Nov.). By C. M. Yonge, FRS. Pp. 453-527. UK £4.30; Overseas £4.45. Vol. 277. No. 951: The Functional Anatomy of the Mantle Complex and Columellar Muscle of Tectibranch Molluscs (Gastropoda: Opisthobranchia), and Its Bearing on the Evolution of Opisthobranch Organization. By R. C. Brace. Pp. 1-56 + plate 1. UK £3.55; Overseas £3.65. (London: The Royal Society, 1977). [172]
- Department of Energy. Energy Paper No. 16: Solar Energy—Its Potential Contribution Within the United Kingdom. (A Report prepared for the Department of Energy by the Energy Technology Support Unit, Harwell.) Pp.viii+81. (London: HMSO, 1977). £3. [172]
- Report of the Centre for Overseas Pest Research, January-December 1975. Pp.vi+142. (London: Centre for Overseas Pest Research, Wrights Lane, W8, 1976). £2.75. [182]
- The Commonwealth Foundation. The First Ten Years, 1966-1976. Pp.97. (London: The Commonwealth Foundation, Marlborough House, SW1, 1976). [182]
- Soil Erosion in the United Kingdom: Field Studies in the Silsoe Area, 1973-75. By R. P. C. Morgan. (Occasional Paper No.4). Pp.41. (Silsoe, Bedford: National College of Agriculture Engineering, Cranfield Institute of Technology, 1977). £2.50. [212]
- Tiem and Us in Science. By Ian Gibson. Pp.30. (Norwich: Dr. I. Gibson, University of East Anglia, School of Biological Sciences, 1977). 30p. [212]
- Health and Safety: Industry and Services, 1975. (A report of the work of HM Factory Inspectorate incorporating the Annual Reports of HM Inspector of Railways and HM Inspector of Explosives.) Pp.92. (London: HMSO, 1977). £2. [212]
- Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 284. No. 1321: Station and Suspension Trajectories of Solid Grains in a Water Stream. By J. E. Abbott and J.R.D. Francis. Pp.225-254. UK £1.80; Overseas £1.90. Vol. 284. No. 1322: Evolving Miogeanticlines of the East Mediterranean (Hellenic, Calabrian and Cyprus Outer Ridges). By A. H. Stride, R. H. Belderson and N. H. Kenyon. Pp. 255-285. UK £3.65; Overseas £3.75. Vol. 284. No. 1323: The Scattering of Sound by a Simple Shear Layer. By D. S. Jones, FRS. Pp. 287-328. UK £2.50; Overseas £2.60. (London: The Royal Society, 1977). [222]
- British Council for Ageing. Research in Gerontology: Problems and Prospects. Pp. 36. (London: Bedford Square Press of the National Council of Social Service, 26 Bedford Square, WCI, 1976). £1 [232]
- Proceedings of the Royal Irish Academy. Vol. 76, Section A, No. 19: On Simple Rings With Involution. By F. Gaines. Pp. 191-193. 32p. Vol. 76, Section B, No. 27: Mycology and Plant Pathology in Ireland. By A. E. Muskett. Pp. 393-472+plates 15-19. £3.80. Nos. 28, 29 and 30: Zeolitic Carbon Dioxide and Ammonia in Y-Type Molecular Sieves. I-Sorbents and Isotherms. II-Affinities, Heats and Entropies of Occlusion III-Models and Equations from the Sorbed Phase. By B. Coughlan and J. J. McEntee. Pp. 473-533. £1.80. No. 31: Some Observations on the Size of the Sponges *Grantia compressa* (Fabr.) and *Sycon ciliatum* (Fabr.) in Strangford Lough, Co. Down. By P. J. S. Boaden, R. J. O'Connor and R. Seed. Pp. 535-542. 52p. Nos. 32, 33 and 34: The Nitrate Assimilation Capacity of Some Irish-Grown Wheat (*Triticum vulgare*) Varieties. I-Levels of Nitrate Reductase Activity and Its Distribution in the Plant. II-An *in vitro* Assessment of Nitrate Reductase Activity and Its Relation to Productivity. III-An *in vivo* Assessment of Nitrate Reductase Activity and Its Relation to Productivity. By M. L. Reilly. Pp. 543-576. £1.32. (Dublin: Royal Irish Academy, 1976). [242]
- IN-519 Cast Chromium-Nickel-Niobium Heat-Resisting Steel—Engineering Properties. (Database No. 4383). Pp. 12. (London: Reader Service, Inco Europe, Ltd., Thames House, Millbank, 1976). gratis. [242]
- University of Strathclyde. Annual Report, 1975/76. Pp. 123. (Glasgow: University of Strathclyde, 1977). [282]
- Imperial College of Science and Technology. Annual Report, 1975/76. Pp.v+44. (London: Imperial College of Science and Technology, 1977). [282]
- Friends of the Lake District. News Letter, Spring 1977. Pp. 27. (Kendal, Cumbria: Consultant Secretary Geoffrey Berry, 27 Greenside, 1977). [282]
- CERN-European Organization for Nuclear Research. CERN 76-21: Guide 7—a General Program for Evaluating the Properties of Scintillation and Cerenkov Counter Optical Systems. By T. Massam. Pp.v+45. (Geneva: CERN, 1976). [12]
- 1976-1977 Directory of Physics and Astronomy Staff Members: North American Colleges and Universities/ Federally Funded Research and Development Centers/ Government Laboratories. Pp.v+293. (New York: American Institute of Physics, 335 East 45 Street, 1976.) \$17.50. [22]
- United States Department of the Interior: Geological Survey. Bulletin 1422-A: Changes in Stratigraphic Nomenclature by the US Geological Survey, 1975. By George V. Cohee and Wilna B. Wright. Pp.iii+84. (Washington, DC: US Government Printing Office, 1976). [32]
- Report of the Finnish Geodetic Institute. 76-3: A Theoretical Analysis of Optical Receivers Used in Satellite Ranging. By A. B. R. Sharma. Pp. 27. 76-6: Lunette de Galilée pour Contrôle du Faisceau Laser. Par L. Oterma. Pp. 5. (Helsinki: Finnish Geodetic Institute, 1976). [32]
- United States Department of the Interior: Geological Survey. Professional Paper 867-B: Stratigraphy of Post-Paleozoic Rocks and Summary of Resources in the Carlin-Pinon Range Area, Nevada. By J. Fred Smith, Jr., and Keith N. Letner. Pp.iv+48+plate 1. (Washington, DC: US Government Printing Office, 1976). [42]
- CERN: European Organization for Nuclear Research. CERN-76-23: Low-Energy Recoil Measurements in High-Energy Physics. By T. Ekelöf. Pp.v+40. Geneva: CERN, 1976). [72]
- Energy, Mines and Resources Canada. Geological Survey of Canada. Bulletin 270: Jurassic Stratigraphy and History of North-Central British Columbia. By H. W. Tipper and T. A. Richards. Pp. 73. \$4.20. Bulletin 274: Middle Devonian Cystiphyllid Corals from the Hume Formation, Northwestern Canada. Pp. 80. \$6. (Ottawa: Geological Survey of Canada, 1976). [72]
- On the Habitability of Mars: An Approach to Planetary Ecosynthesis. Edited by M. M. Averbner and R. D. MacElroy. Prepared by Ames Research Center. (NASA SP-414). Pp. xi+105. (Washington, DC: Scientific and Technical Information Office, National Aeronautics and Space Administration, 1976. For sale by the National Technical Information Service, Springfield, Virginia, 22161.) \$5.25. [72]
- American Museum of Natural History. 107th Annual Report, July 1975, through June 1976. Pp. 56. Research Report and Bibliography, July 1975 through June 1976. Pp. 30. (New York: American Museum of Natural History, 1976). [72]
- Diving-Dykning. Nr. 1, Vol. 1, 1977. Pp. 1-20. Edited by George Ballantine. (5985 Soebø, Aeroe Island, Denmark: Association of Danish Divers, 1977). [82]
- Geological Survey of Canada. Physiography, Eastern Canada and Adjacent Areas. (4 sheets). Scale 1:2,000,000. Compiled by B. V. Sanford and G. M. Grant. (Ottawa: Department of Energy, Mines and Resources, 1976.) \$6. [82]
- Energy, Mines and Resources Canada. Geological Survey of Canada. Paper 77-1A: Report of Activities, Part A. Pp. viii+527. (Ottawa: Geological Survey of Canada, 1977.) \$7.20. [92]
- United States Department of the Interior: Geological Survey. Bulletin 1278-D: Geochemical Exploration Techniques Based on Distribution of Selected Elements in Rocks, Soils, and Plants, Mineral Butte Copper Deposit, Pinal County, Arizona. By Maurice A. Chaffee. Pp. iv+55. Professional Paper 743-E: Stratigraphic Distribution of Species of the Megaspore Genus *Minerisporites* in North America. By Robert H. Tschudy. Pp. iii+10+4 plates. Professional Paper 1001: Numerical Simulation Analysis of the Interaction of Lakes and Ground Water. By Thomas Winter. Pp. iv+45. (Washington, DC: US Government Printing Office, 1976). [92]
- World Armaments—Abundance Amid Scarcity. Pp. 17. (Stockholm: Stockholm International Peace Research Institute, 1976). [102]
- Annals of the South African Museum. Vol. 72, Part 5: Upper Cretaceous Ammonites from a Borehole Near Richards Bay, South Africa. By Herbert Christian Klinger and William James Kennedy. Pp. 69-107. £3.50 Vol. 72, Part 6: New Procolophonids from the Triassic *Cynognathus* Zone of South Africa. By C. E. Gow. Pp. 109-124. R. 2.20. Vol. 72, Part 7: Larval Development of *Sardinops ocellata* (Pisces: Clupeidae). By Elizabeth Louw and M. J. O'Toole. Pp. 125-145. R. 2.50. (Cape Town: South African Museum, 1977). [102]
- United States Department of Agriculture. Index-Catalogue of Medical and Veterinary Zoology. Supplement 21, Part 1, Authors: A to Z. By Shirley J. Edwards, Judith H. Shaw, Martha W. Hood, Jane D. Rayburn, Margie D. Kirby and Deborah A. Tolson. Pp. xv+531. (Washington, DC: US Government Printing Office, 1976). [102]
- New Zealand: National Roads Board. Road Research Unit Bulletin No. 30: Analysis of a Twin Spine Box-Girder Model. By M. J. N. Priestley. Pp. 46. (Wellington, NZ: National Roads Board, 1975). [102]
- CERN—European Organization for Nuclear Research. CERN 77-01: A New Position-Sensitive Detector for Thermal and Epithermal Neutrons. By A. P. Jeavons, N. L. Ford, B. Lindberg and R. Dachot. Pp. 9. (Geneva: CERN, 1977). [102]
- Unesco. A Comprehensive Plan for the Global Investigation of Pollution in the Marine Environment and Baseline Study Guidelines. (Intergovernmental Oceanographic Commission Technical Series, No. 14.) Pp. 42. (Paris: Unesco, 1976). [112]
- United States Department of the Interior: Geological Survey. Professional Paper 933: Geology and Resources of Fluorine in the United States. Edited by Daniel R. Shawe. Pp. vi+99. (Washington, DC: US Government Printing Office, 1976). [112]
- Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture. Rapport Annuel, Exercice 1975. Pp. 531. (Bruxelles: IRSIA, 1976). [142]
- World Health Organization. International Classification of Diseases for Oncology (ICD-O). Pp. xxiii+131. (Geneva: WHO; London: HMSO, 1976). Sw.fr.20; \$8. [142]
- Water—The Essential Resource. By Philip W. Quigg. Pp. 20. (New York: National Audubon Society, 950 Third Avenue, 1977). [142]
- United States Department of the Interior: Geological Survey. Bulletin 1421-A: Physical Properties of the Coexisting Phases and Thermochemical Properties of the H₂O Component in Boiling NaCl Solutions. By John L. Haas, Jr. Pp. v+73. (Washington, DC: US Government Printing Office, 1976). [162]
- Wattle Research Institute. (South Africa Wattle Industry; South African Timber Growers' Association, University of Natal; State Department of Forestry; Forestry Council.) Report for 1975-1976. (Twenty-Ninth Year). Pp. 111. (Pietermaritzburg: Wattle Research Institute, University of Natal, 1976). [162]
- The Biogeography and Numerical Taxonomy of the Oegopsid Squid Family Ommastrephidae in the Pacific Ocean. By John H. Wormuth. (Bulletin of the Scripps Institution of Oceanography, Vol. 23.) Pp. 90. (Berkeley, Los Angeles and London: University of California Press, 1976.) \$3.50. [172]
- Carnegie Institution. Annual Report of the Director of the Department of Terrestrial Magnetism, 1975-1976. Pp. 109-272. (Reprinted from *Carnegie Institution Year Book* 75.) (Washington, DC: Carnegie Institution, 1976). [182]
- Meddelelser fra Danmarks Fiskeri- og Havundersøgelse. N.S. Vol. 7: A Markovian Decision Process Applied to Optimization of Production Planning in Fish Farming. By Per Sparre. Pp. 111-197. N.S. Vol. 7: Elvers, *Anguilla anguilla* and *Anguilla rostrata* from Two Danish Localities. Size, Body Weight, Developmental State and Number of Vertebrae Related to Time of Ascent. By Jan Boetius. Pp. 199-220. (Copenhagen: The Danish Institute for Fishery and Marine Research, 1976). [212]
- International Classification of Diseases for Oncology (ICD-O). Pp. xxiii+131. (Geneva: WHO; London: HMSO, 1976). Sw.fr.20; \$8. [212]
- US Department of the Interior: Geological Survey. Bulletin 1421-B: Thermodynamic Properties of the Coexisting Phases and Thermochemical Properties of the NaCl Component in Boiling NaCl Solutions. By John L. Haas, Jr. Pp. v+71. Professional Paper 887: Mercurite—a Complex Assemblage of Chromium Minerals from Guyana. By Charles Milton, D. E. Appleman, et al. Pp. vi+29+plates 1-6. (Washington, DC: US Government Printing Office, 1976). [212]
- Report of the Government Chemical Laboratories, Western Australia, for the year 1975. Pp. 38. (Perth, WA: Government Chemical Laboratories, 1976). [212]
- World Health Organization. Public Health Papers, No. 62: An Environmental Sanitation Plan for the Mediterranean Seaboard: Pollution and Human Health. By J. Brisou. Pp. 96. (Geneva: WHO; London: HMSO, 1976). Sw.fr.10; \$4. [212]
- FAO, Rome. Cadmium, Lead, Mercury and Methylmercury Compounds: a Review of Methods of Trace Analysis and Sampling with Special Reference to Food. By Pieter L. Schuller and Harold Egan. Pp. viii+97. £3.52. FAO Fisheries Technical Papers. No. 142: The Production of Fish Meal and Oil. Pp. x+54. £2.27. No. 150: Manual of Methods in Aquatic Environment Research. Part 2: Guidelines for the Use of Biological Accumulators in Marine Pollution Monitoring. Edited by J. E. Portman. Pp. viii+76. £2.92. (Rome: FAO; London: HMSO, 1975 and 1976). [222]
- CSIRO, Australia. Division of Food Research—Report of Research, 1975/1976. Pp. 119. (Sydney: CSIRO, 1976). [232]
- CSIRO, Australia. Annual Report of the Division of Entomology, 1975/1976. Pp. 120. (Canberra, ACT: CSIRO, 1976). [232]
- Smithsonian Contributions to Zoology, No. 228: A Review of the Systematics and Zoogeography of the Freshwater Species of *Palaemonetes* Heller of North America (Crustacea: Decapoda). By Ned E. Strelnik. Pp. 27. (Washington, DC: Smithsonian Institution Press, 1976. For sale by US Government Printing Office.) [242]
- United States Department of the Interior: Geological Survey. Bulletin 1432: The Reinhardt Thiessen Coal Thin-Section Slide Collection of the U.S. Geological Survey—Catalog and Notes. By James M. Schopf and Orrin G. Oftedahl. Pp. iv+58. Water-Supply Paper 2040: Ground Water in the Harrisburg-Halsey Area, Southern Willamette Valley, Oregon. By F. J. Frank. Pp. iv+45+1 plate. (Washington, DC: US Government Printing Office, 1976). [252]
- Organization for Economic Co-operation and Development. Paris. Environment Directorate. Study on Economic and Policy Instruments for Water Management. Water Management in France. Pp. 65. Water Management in the Federal Republic of Germany. Pp. 55. Water Management in Japan. Pp. 49. Water Management in the Netherlands. Pp. 50. Water Management in Canada. Pp. 61. Water Management in the United States. Pp. 127. Water Management in Finland. Pp. 33. Second Report on the Problem of Photochemical Oxidants and Their Precursors in the Atmosphere. Pp. 103. Water Management in the United Kingdom. Pp. 41. (Paris: OECD, 1976). [282]
- Guide to the Collection and Transport of Virological Specimens. By C. R. Madeley. Pp. 39. (Geneva: WHO; London: HMSO, 1977). Sw.fr. 10; \$4.20. [282]
- CERN—European Organization for Nuclear Research. CERN 77-02: An INTEL 8080 Microprocessor Development System. By P. J. Horne. Pp. v+20. (Geneva: CERN 1977). [282]

Other countries—February

The Coconut Industry Board, Jamaica. 15th Report of the Research Department, July 1974/December 1975. Pp. 55. (Kingston, Jamaica: Coconut Industry Board, PO Box 204, 1976.) J\$3; £2; US\$3. [12]